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SYSTEMATICS OF CACTACEAE JUSS.:

PHYLOGENY, CPDNA EVOLUTION, AND CLASSIFICATION,

WITH EMPHASIS ON THE GENUS *MAMMILLARIA* HAW.

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Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw.

by

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Dedication

For my father, Bruce Crozier.

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The first cactus collected for this study, *Neolloydia conoidea*, was plucked under the watchful eye of Barton Warnock long before I ever thought of pursuing academic botany. Barton loved the Trans Pecos and its plants. He patiently answered an untold number of beginners' questions, insisted you weren't a very good botanist if you had to ask permission to collect a plant, and highly recommended 5-ply tires. He disdained government "interference" in his botanical activities but accepted the environmental center they named after him. He used to call me "Sunshine" and perhaps it is from him I got the idea that botany is fun and a worthwhile pursuit!

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Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw.

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The Cactaceae is a family of New World dicotyledonous angiosperms noted for its ecogeographic significance and highly specialized vegetative features adapted to arid environments. Generic limits and phylogenetic relationships between taxa of this family have been in a state of flux, hindering evolutionary studies. In this thesis original DNA sequence data from the multiple chloroplast regions for 157 species of Cactaceae, Portulacaceae, Basellaceae, Halophytaceae, and Didieraceae are analyzed using maximum parsimony, minimum evolution, and Bayesian methods to reconstruct phylogenetic relationships, and shed light on the tempo and mode of Cactaceae evolution. The seemingly abrupt appearance of novel adaptations marking the origin of new taxa at relatively higher rank has intrigued biologists even before Darwin. In Cactaceae, major morphological disjunctions between leafy and leafless cacti have been difficult to explain. Evidence of lineage specific increases in the rate of accumulation of nucleotide replacements is presented here based on Bayesian analyses of three protein-coding chloroplast genes. The first increase in evolutionary rate, occurring soon after the origin

of the family, is discussed in the context of aridity as a stimulus to quantum evolution. A much more recent rate increase was observed in the derived genus *Mammillaria*. *Mammillaria,* the largest genus of Cactaceae, has historically been viewed as the main lineage in a complex of small and micro genera sharing tuberculate podaria and complex taxonomy. This study also investigates "What is a *Mammilllaria*?" To circumscribe a monophyletic *Mammillaria* and clarify several small genera, most type species of the *Mammillaria* complex were sampled and character sampling was expanded to include four intergenic spacer regions and three Group II chloroplast introns in addition to the 3-gene coding data set. In contrast to previous molecular studies in Cactaceae, the broad sampling of taxa and characters used here provided sufficient resolution and confidence in results to allow revision of the suprageneric classification based on chloroplast phylogeny. A nomenclaturally sensitive approach has been used in producing an explicitly phylogenetic revision of classification.

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Chapter 1: Introduction: *Studies in Cactaceae*

The Cactaceae is a family of New World trees, shrubs, lianas, and globular, cylindrical-columnar, or laminar stem succulents noted for highly specialized vegetative features and adaptation to arid environments. Icons of the American deserts, the climatic spectrum they inhabit is actually very broad. Although most species inhabit semi-arid habitats, cacti range from arid areas of no measurable rainfall, as in the Atacama Desert where they survive as geophytes, to tropical rainforests of 500 cm annual precipitation where they adapted to relatively drier microhabitats as epiphytes (Taylor, 1997; Rebman & Pinkava, 2004). Their altitudinal range extends from below sea level to snowy peaks of more than 5000 meters in the Andes. Body size variation is equally impressive, encompassing columnar species 20 meters tall or more, almost a meter in diameter and weighting tons (*Pachycereus, Carnegiea, Trichocereus*), to miniature forms less than a single centimeter in diameter that persist drought conditions as flat disks weighing less than a pea (*Blossfeldia*).

The biology of cactus adaptations and concomitant implications for the evolution of plant responses to stress and climate change naturally attracts ecological and evolutionary study. Numerous studies have investigated mechanisms that allow cacti to persist under conditions of high temperatures and little water: e.g., water storage via anatomical adaptations promoting stem succulence, limiting water loss via cuticular

thickening, hairs, and spines, the adaptation for nighttime respiration/daytime assimilation by Crassulacean acid metabolism, and the water relations important for tolerance to radiation and high temperatures (e.g. Walter, 1971; Walter & Stadelman, 1974; Barthlott, 1977; Gibson & Nobel, 1986; Buxbaum, 1951). Although many genera have not been studied, the flowers of cacti (classified as bee, bat, or hummingbird pollinated; Porsch 1938, 1939; Grant & Grant, 1979) exhibit a low pollen to ovule ratio even for zoophilous plants, and this is also presumed to be an adaptive advantage in stressful environments by conserving energy placed into the male gametophyte (Linskens, 1983; Nassar et al., 1997; Pimienta-Barrios & Castillo, 2002). Asynchronous fruit and floral development and lack of seed dormancy common in Cactaceae is yet another advantage in arid environments where rainfall is erratic (Pimienta-Barrios & Nobel, 1995). However, lack of a robust phylogenetic hypothesis for the family and stable cladistic circumscription of its component taxa, essential tools for comparative study, has hindered progress in evolutionary studies of adaptation. The studies of Cactaceae presented in the following chapters bring new chloroplast DNA data to bear on the reconstruction of phylogeny for this interesting and diverse group, beginning with a generic level survey of the family in Chapter 2.

The precise number of genera and species comprising the Cactaceae remains unknown despite the group's broad popularity. According to Angiosperm Phylogeny Group webpage (Stevens 2001 on [2005]) the number of genera in the family varies by a factor of ten and the number of species by a factor of two depending on the author. However, more than thirty years of collaborative work by an international consortium (I.O.S.[1]) has culminated in a checklist (Hunt, 1990, 1999; reports by Hunt and Taylor,

[1] International Organization for Succulent Plant Research.

1986, 1990) that recognizes 108[2] genera and 1306 species with another 582 species provisionally accepted. This list serves as a benchmark adopted by governmental agencies in many countries enforcing the current international treaty intended to regulate commercial trade of endangered species (C.I.T.E.S.[3]). Even so, many determinations still remain to be tested with well-sampled molecular phylogenetic studies. A few are challenged by the results presented in Chapter 2. In Chapter 3 the generic boundaries of *Mammillaria,* the largest genus of Cactaceae*,* are examined with respect to delimitations of several closely related small genera.

Cronquist (1988) noted Cactaceae as one of the few large dicot families with clear ecogeographic significance. The family is endemic to the New World[4] distributed from Canada to Patagonia almost to the Straits of Magellan, most common trans-equatorially between the 35th latitudes, but conspicuously absent from the Amazon Region (Barthlott, 1979). A few genera extend to the Galapagos Islands in the Pacific and Fernando de Noronha in the Atlantic. One species, *Rhipslis baccifera*, is distributed in Africa and Sri Lanka. Many species are highly restricted endemics. Of the three geographical centers of diversity and endemism that are recognized for Cactaceae, Megamexico[5] is outstanding. Here nearly half the species of the family are native, and more than ¼ of the (I.O.S. accepted) genera and 1/3 of species are endemic (Taylor, 1997). *Mammillaria* is a derived member of the clade of endemic dwarf cacti that contains many highly localized endemics with specialized growth forms and whose ancestors were probably first to arrive in North America perhaps by long distance dispersal. The phylogenetic trees on the following pages clearly illustrated the multiplicity of cactus invasions into North

[2] Not including hybrid genera; this number is a conservative count.
[3] Convention on International Trade in Endangered Species.
[4] With the exception of *Rhipsalis baccifera*.
[5] The nation of Mexico plus the southern portions of Texas, New Mexico, Arizona and California along with the Guatemalan Highlands.

America from six major cactus lineages: 1. *Pereskia,* 2. Opuntioids: represented by *Opuntia* and *Consolea* as well as *Cylindropuntia* and *Pereskiopsis*, 3. *Rhipsalis*, 4. Melocactus, 5. Pachycereeae and epiphytic Hylocereae, and 6. Cactoideae, the so-called the North American dwarf cactus lineage.

Despite its important centers of endemism in the central Andes and eastern Brazil, the southern subcontinent does not contain the diversity of highly modified growth forms as in North America, with the exception of *Blossfeldia* (Taylor, 1997). This unusual monotypic miniature was identified by the studies in Chapter 2 as the only extant link between the clearly monophyletic cactoid cacti and the leaf-bearing lineages of Cactaceae comprising *Pereskia*, *Maihuenia,* and the opuntioids (*Opuntia* and its allies). Deserving of special status, *Blossfeldia* is segregated from the clearly monophyletic clade Rhipsalidoideae whose members share the loss of the *rpo*C1 intron and a large deletion in the *trn*T-*trn*L intron (Wallace & Cota, 1996; Applequist & Wallace, 2002; Stevens, 2001 onward [2005]) based on results of phylogenetic analysis presented in Chapters 2, 4, and 5. Chapter 5 includes a now published manuscript resulting from preliminary molecular work that describes a new subfamily, Blossfeldioideae Crozier, in the context of these data and past division of the group into subfamilies and provides a key to the subfamilies of Cactaceae.

FAMILY ORIGINS

A derived member of the Caryophyllales, Cactaceae exhibit the distinctive characteristics of that group (Bittrich, 1993) including the embryological syndrome, campylotropous ovule and presence of starchy perisperm replacing endosperm, (for which the order was named Centrospermae by Eichler in 1878), as well as 3-celled pollen. At least 15 molecular surveys from both nuclear and chloroplast genomes (summarized in Cuenoud et al., 2002) definitively place the Cactaceae with the core

Caryophyllales. This is also supported by micromorphological evidence, specifically the unique presence of proteinaceous sieve-element plastids, and phytochemical studies that show betalain pigments have replaced anthocyanins in most metabolic processes.

Ehrendorfer (1976) characterized the diversification and radiation of the Caryophyllales as one of the most fascinating in the plant kingdom. He hypothesized the origins of Cactaceae and Portulacaceae from an ancestor selected for anemophily in pollinator-poor, warm, windy, arid or semi-arid open habitats of the late Cretaceous. Ehrendorfer postulated a reversal to zoophily in derived lineages Cactaceae and Portulacaceae, marked by the development of brilliant showy flowers, secondary polyandry and betalain pigments, resulting from selection driven by an increase of pollinators, either by expansion of the plants into more mesic pollinator-rich habitats, or by the movement of more discriminating pollinators into arid zones.

The sister group of Cactaceae appears to be Portulacaceae sensu stricto (Thorne 1983; Gibson and Nobel, 1986; Hershkovitz, 1991; Downie & Palmer, 1994; Gibson, 1994; Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001b). Results presented in Chapter 2 based on limited sampling from Didieraceae, Basellaceae, Portulacaceae, and Halophytaceae, the 'succulent clade' (Manhart and Rettig, 1994) or Portulacinae Thorne (Cronquist and Thorne, 1994), are consistent with this hypothesis. However, relationships among Cactaceae's closest relatives, including Hectorellaceae, are still a matter of debate. Moreover, evidence suggests that Portulacaceae as currently recognized may well be paraphyletic (Carolin 1987, 1993; Rodman, 1990; Hershkovitz & Zimmer, 1997; Applequist & Wallace, 20001b).

Portulacaceae is also renowned for its variation in chromosome numbers, a condition that lends little assistance to those who would attempt to reconstruct the origin of the x=11 base chromosome number in Cactaceae. Turner (1994) suggested that the

Cactaceae base number may have evolved from $\underline{x}$=12 via aneupoloid loss, and this remains the best hypothesis since Basellaceae, Didieraceae, and the order as a whole share the *x*=12 base number. Within the Cactaceae aneupoloidy, cytomixis, inversions, translocations, secondary association, and extra nuclear bodies have been observed (Ross, 1981; Pinkava et al., 1985). However, polypoloidy is by far the most common form of chromosomal variation, primarily important in the subfamily Opuntioideae and apparently not occurring in *Pereskia* or *Maihuenia* (Rebman & Pinkava, 2001).

The Cactaceae have long been considered natural based on morphology. Their highly modified stem is easily recognized by the areole, a spine and/or flower bearing short shoots delimited by a cushion-like indumentum. The monophyly of Cactaceae is supported by molecular evidence (Downie & Palmer, 1993; Hershkovitz & Zimmer, 1997; Applequist & Wallace, 2001) and the family is one of the best circumscribed among those most closely related including Portulacaceae, Basellaceae, Didieraceae, Halophytaceae, Hectorellaceae, and Molluginaceae.

HISTORY AND CLASSIFICATION

The earliest collections of cacti were brought into cultivation in Europe as curiosities or medicinal resources rather than as herbarium specimens. European botanical descriptions first appear in the late 16th Century by which time cacti were grown in many countries.

Although some species undoubtedly remain to be discovered, the species of cacti are now fairly well known and delimited. More difficult has been the circumscription of genera, as Chapter 3 highlights. This is perhaps because the family is apparently quite young but also because in many cases characteristics shared by historical descent cannot be distinguished from those due to shared ecological response. The Cactaceae is

infamous for the number of morphological features that have arisen in parallel or thought to have reversed character polarity.

Until the advent of molecular systematics, the relationships among tribes and subfamilies of Cactaceae could not be clearly ascertained. Evolutionary parallelisms of morphological features have obscured relationships between major groups of cacti. Berger's partial dendrograms (1926) show his attempt to connect genera in evolutionary lineages, and Barthlott's (1988) diagram in the style of Dahlgren made less than twenty years ago showed a hypothesis of tribal circumscription and of close relationship between Notocacteae (including Trichocereeae), Cereeeae, and Browningieae, but only vaguely depicted relationships among other tribes. The latest generic synopsis (Barthlott & Hunt, 1993) adds little to our understanding of tribal relationships. In contrast, numerous molecular synapomorphies precisely define many clades within Cactaceae.

The need for an explicitly phylogenetic framework for evolutionary studies and the emerging evidence of natural clades within the family demands a fresh look at classification. Recognition of large morphological discontinuities has been the basis of the long-standing division of the family into only three main groups. Now the simplicity of the troika scheme is challenged by molecular evidence.

In Chapter 4 a revised suprageneric classification of Cactaceae is presented informed by results of chloroplast DNA studies in Chapters 2 and 3. It is understood that gene trees and organismal trees may not entirely coincide. However, new data provide a much better approximation than has been available and precipitate a novel classification scheme upon which a new debate can begin. Every effort has been made to make this classification sensitive to nomenclatural precedents so that phylogenetic and traditional systems are not in conflict.

WHY IS CACTUS SYSTEMATICS SO PRICKLY?

Adaptation has been at the heart of evolutionary theory since the earliest days of the science. Evolution played out on the ecological stage is more complex than mathematical drift alone can predict. The Cactaceae provides a case in point (no pun intended). Although much has been gained through modeling neutral evolution, not all evolution is neutral, and the molecular basis for most morphological traits remain largely undiscovered. Through comparative study, not only of taxa, but also of the DNA itself, footprints of the adaptation process may still be observable. In the next chapter comparison of three chloroplast coding regions illustrates that the nucleotide substitution process has changed over time in Cactaceae, presenting unique problems for reconstructing phylogeny and modeling processes of nucleotide evolution. Since rate changes in Cactaceae appear in conjunction with the family's two largest species radiations and at the inception of adaptive traits for which the family is renown, selective forces are suspected. These preliminary results offer exciting prospects for future studies in this unique group of plants.

Chapter 2: Tempo and Mode of Cactus Evolution: *Insights from chloroplast DNA sequence data*

INTRODUCTION

Cactaceae are characterized by highly specialized stem and leaf morphology as well as anatomy assumed to be the result of adaptation to arid environments. Stems of cacti bear unique proleptic short shoots felted with trichomes, termed areoles, that in turn bear spines that are highly modified leaves (Buxbaum, 1951c; Boke 1954; Leuenberger, 1986). Although some members of other families, e.g., Portulacaceae and Didieraceae, may also bear short shoots and trichomes, the cushion-like indumentum delimiting the cactus areole is distinctive enough to serve as the major recognition character for the group. The stem-character of the cactus receptacle is also unusual in that axillary meristems may remain active, the receptacular areoles of some species even giving rise to axillary shoots as well as trichomes and spines. Cacti all share a 6 kilobase inversion in the large single copy region of the chloroplast genome (Downie & Palmer, 1994) and the monophyly of Cactaceae has been supported by previous molecular phylogenetic studies using nuclear ribosomal ITS (Hershkovitz & Zimmer, 1997) and chloroplast *ndh*F (Applequist & Wallace, 2001b). Primarily a New World family, comprising some 1500 accepted species (Hunt, 1999), Cactaceae are marked by a great diversity of growth form that accompanies the broad climatic spectrum and range of microhabitats that cactus species inhabit.

Stem succulence and lack of persistent broad/laminar leaves dominate the family except for the basal genus *Pereskia* (17 species). Traditional division of the family into subfamilies Pereskioideae, Opuntioideae, and Cactoideae reflects major discontinuities in Bauplan as well as seed and other characters (Barthlott, 1988; Leuenberger, 1997; see

also Chapter 5). The Cactoideae is an essentially leafless complex circumscribing nearly 70% of species of the family. Stems of Opuntioideae tend to be segmented and bear terete, deciduous, usually small leaves. The group is considered highly derived due to several very divergent characters lacking clear transition series, among them a seed entirely encased in a bony aril, the presence of glochids (tiny barbed spines deciduous to the touch), retrorse scabrose spines and a unique stomatal morphology (Stuppy, 2002). The phylogenetic origins and affinities of Opuntioideae are not obvious. In fact, the absence of transitional forms between subfamilies has confounded efforts to infer their relationships based on morphology, and to understand the evolution of stem succulence and other modifications leading to Opuntioideae and Cactoideae from ancestors presumably less modified and more typically dicotyledonous as in *Pereskia*.

The seemingly abrupt appearance of novel adaptations marking the origin of new taxa at relatively higher rank [6] has intrigued biologists from the origins of evolutionary thought even before Darwin (e.g., Lamark and St. Hilaire cf. Nordenskiöld, 1928 in Wright, 1982). Simpson (1944, 1953) recognized the pattern of such origins, preceded by gaps in the paleontological record, not as an artifact of the record but as a problem of evolutionary rates. He postulated that differences in rate, not only divergent evolution at comparable rates, was an important contributor to the great diversity of organisms on earth. Quantum evolution was described by Simpson (1944) as the rapid evolutionary invasion of a new adaptive zone driven by natural selection in small populations. This idea borrowed from Wright's (1932) shifting balance theory that established (by contribution of a mathematical model) the paradigm of an adaptive landscape. According to Simpson a tachytelic (rapid) evolutionary rate distribution (of phenotypic characters)

[6] G.L. Stebbins, Jr., G.G. Simpson, and other figures of the "modern synthesis" in evolutionary biology theorized the biological reality and origins of categories higher than the rank of species.

accompanied a population shift from one adaptive peak to another at the origin of higher categories of organisms.

Precisely which mechanisms lead to such quantum evolutionary shifts, to the characters diagnostic of higher taxa, and which factors govern evolutionary rates have been debated ever since the modern synthesis in evolutionary biology. Population geneticists now mostly agree that these changes can be explained by standard microevolutionary forces such as selection, mutation, drift, and recombination (Lande, 1986) though a few (Goldschmidt, 1940; Gould, 1977) have suggested that processes distinct from those occurring within species were needed to explain the phenomenon. Modeling quantum shifts for gene frequencies Wright (1932) emphasized the availability of new ecological niches as the determining factor for rapid change. Building on the fitness topography for phenotypic characters derived by Lande (1976), and extended by Felsenstein (1979) for polygenic characters, Kirkpatrick (1982) modeled continuous characters during an adaptive shift and showed that neither genetic drift nor environmental change was essential for large abrupt shifts. However, in Kirkpatrick's model, rapid evolution is initiated when the population equilibrium is perturbed either by environmental changes, or by an internal change in a character that increases phenotypic variance. The interaction of genetic mechanisms with spatial or ecological factors figures prominently in some theories. For example, the importance of speciation (reproductive isolation arising in geographically isolated populations) in the sudden appearance of evolutionary novelties was stressed by Mayr (1954, 1963, 1970) and is central to the theory of punctuated equilibrium (Eldgredge & Gould, 1972).

Aridity has been suggested as a stimulus to plant evolution that may initiate large adaptive shifts (Stebbins, 1952, 1959; Axelrod, 1967). Evolution resulting from parallel selective regimes was inferred from the observation of similar life forms occurring in

parallel dry vegetation zones; parallel and adaptive features in distantly related taxa indicated the presence of a long-term selective regime. The parallel evolution of stem succulent Cactaceae in the Americas and stem succulent Euphorbiaceae in Africa is a well-known example. The zonation of similar adaptive types was also observed corresponding to zones of dry habitats north and south of moist tropical rain forest, savanna, dry deciduous forest, tropical desert grading into sclerophyll woodlands and scrub (Axelrod, 1967). Within Cactaceae equally significant parallelisms occur, for example, columnar and globular forms, and epiphytic habit evolving in both North and South America. Drought as a selective pressure on multiple characters concurrently is expected to result in rapid evolution; this is especially true because topographical differences in landscape contribute to diversification and fragmenting of populations near species borders more where moisture is limited (Stebbins, 1952).

Cactaceae are important components of the evolution of desert floras in the Americas; they are the dominant species in some plant communities. It is of intrinsic interest to biologists to have a robust estimate of evolutionary relationships for any comparative study, and especially for investigations of parallel adaptive features. The need for a well-supported phylogeny of Cactaceae at the generic level as well as the need for taxonomic revision is clear.

The first goal of this investigation was to estimate the phylogeny of Cactaceae useful for comparative studies. We sampled three protein-coding regions of the chloroplast genome for 122 taxa from Cactaceae and outgroups and estimated phylogeny by different methods. In the course of phylogenetic analyses, the relative rates of nucleotide substitution across lineages of Cactaceae were also investigated. The tempo of molecular rates in Cactaceae is reviewed in light of a new understanding of cactus relationships based on this study.

MATERIALS AND METHODS

Taxon and Character Sampling

One hundred and thirteen cactus species were sampled, chosen from all subfamilies and tribes (Buxbaum, 1974) to represent the taxonomic diversity of the family (Table 2.1). This represents slightly more than 66% of the genera fully and provisionally accepted by Hunt (1999). Because delimitation of genera in Cactaceae is difficult and subject to broad disagreement, type species and genera were sampled wherever possible so that these results might inform taxonomic revisions. Particular emphasis was placed on sampling the North American taxa. Voucher specimens are deposited in the countries of origin where appropriate, and/or at TEX.

Table 2.1. List of taxa sampled, voucher numbers and countries of origin.

TAXON	VOUCHER #	ORIGIN
Acharagma aguirreana (Glass & Foster) Glass	*Crozier DISS1*	Mexico
Acharagma roseanus (Boedeker) E. F. Anderson	*Crozier DISS2*	Mexico
Alluaudia dumosa Drake	*Crozier DISS16*	Madagascar
Anacampseros kurtzii Bacigalupo	*Crozier DISS17*	Argentina
Aporocactus flagelliformis (L.) Lemaire	*Crozier DISS3*	Mexico
Arequipa rettigii (Quehl) Oehme	*Crozier DISS102*	Peru
Ariocarpus retusus Scheidweiler	*Crozier DISS4*	Mexico
Arrojadoa penicillata (Guerke) Britton & Rose	*Crozier DISS160*	Brazil
Astrophytum myriostigma (Karwinsky ex Zucc.) Lem.	*Crozier DISS6*	Mexico
Austrocactus patagonicus (Weber ex Speg.) Hosseus	*Crozier DISS18*	Argentina
Austrocylindropuntia subulata (Berger) Backeb.	*Crozier DISS19*	Bolivia
Aztekium hintonii Glass & Fitz Maurice	*Crozier DISS7*	Mexico
Aztekium ritteri (Boedeker) Boedeker	*Crozier DISS8*	Mexico
Basella alba L.	*Crozier DISS20*	USA

Blossfeldia liliputana Werdermann	*Crozier DISS21*	Argentina
Browningia candelaris (Meyen) Britton & Rose	*Crozier DISS9*	Peru
Calymanthium substerile Ritter	*Crozier DISS11*	Peru
Carnegiea gigantea (Engelmann) Britton & Rose	*Crozier DISS12*	USA
Cephalocereus senilis (Haworth) Pfeiffer	*Crozier DISS13*	Mexico
Cereus jamacaru DC	*Crozier DISS22*	Brazil
Cintia knizeii Riha	*Crozier DISS14*	Bolivia
Cleistocactus baumannii (Lemaire) Lemaire	*Crozier DISS15*	Brazil
Consolea rubescens (Salm-Dyck) Lemaire	*Crozier DISS23*	Puerto Rico
Copiapoa cinerea (Philippi) Britton & Rose	*Crozier DISS24*	Chile
Copiapoa marginata (Salm-Dyck) Britton & Rose	*Crozier DISS25*	Chile
Corryocactus brevistylus (Schumnann) Britton & Rose	*Crozier DISS26*	Peru
Coryphantha sulcata (Engelmann) Britton & Rose	*Crozier DISS29*	USA
Cylindropuntia imbricata (Haw.) F. Knuth	*Crozier DISS30*	USA
Denmoza rhodocantha (Salm-Dyck) Britton & Rose	*Crozier DISS31*	Argentina
Discocactus placentiformis (Lehmann) Schumann	*Crozier DISS32*	Brazil
Disocactus biformis (Lindley) Lindley	*Crozier DISS33*	Guatemala
Echinocactus platyacanthus Lemaire	*Crozier DISS34*	Mexico
Echinocactus texensis Hopffer	*Crozier DISS35*	USA
Echinocereus viridiflorus Engelmann	*Crozier DISS36*	USA
Echinopsis eyriesii (Turpin) Pfeiffer & Otto	*Crozier DISS37*	Argentina
Echinopsis macrogona (Salm-Dyck) Friedrich & Rowley	*Crozier DISS161*	Bolivia
Epiphyllum phyllanthus (L.) Haworth	*Crozier DISS38*	Brazil
Epithelantha micromeris (Engelmann) Weber	*Crozier DISS39*	USA
Eriosyce islayensis (Foerster) Kattermann	*Crozier DISS40*	Peru
Eriosyce subgibbosa (Haworth) Kattermann	*Crozier DISS41*	Chile
Escobaria tuberculosa (Engelmann) Britton & Rose	*Crozier DISS44*	USA
Escontria chiotilla (Weber ex Schumann) Rose	*Crozier DISS45*	Mexico
Eulychnia breviflora Philippi	*Crozier DISS46*	Chile
Ferocactus wislizeni (Engelmann) Britton & Rose	*Crozier DISS48*	USA
Frailea cataphracta (Dams) Britton & Rose	*Crozier DISS49*	Brazil
Geohintonia mexicana Glass & Ftiz Maurice	*Crozier DISS50*	Mexico
Glandulicactus uncinatus (Galeotti) Backeberg	*Crozier DISS51*	Mexico
Gymnocalycium denudatum (Link & Otto) Pfeiffer ex Miller	*Crozier DISS52*	Brazil
Haageocereus pseudomelanostele (Werdermann & Backberg) Backberg	*Crozier DISS53*	Peru
Halophytum ameghinoi Speg.	*Crozier DISS54*	Argentina
Hatiora salicornioides (Haworth) Britton & Rose ex Bailey	*Crozier DISS55*	Brazil

Hylocereus triangularis (L.) Britton & Rose	*Crozier DISS56*	Jamaica
Leptocereus quadricostatus (Bello) Britton & Rose	*Crozier DISS57*	Puerto Rico
Leuchtenbergia principis Hooker	*Crozier DISS58*	Mexico
Lophophora williamsii (Lemaire ex Salm-Dyck) J. Coulter	*Crozier DISS59*	USA
Maihuenia patagonica (Philippi) Spegazzini	*Crozier DISS60*	Argentina
Maihuenia poeppigii (Pfeiffer) Schumann	*Crozier DISS61*	Argentina
Maihueniopsis glomerata (Haw.) Kiesling	*Crozier DISS62*	Argentina
Mammillaria mammillaris (L.) Karsten	*Crozier DISS77*	Venezuela
Matucana haynei (Otto ex Salm-Dyck) Britton & Rose	*Crozier DISS92*	Peru
Melocactus caroli-linnaei Taylor	*Crozier DISS93*	Jamaica
Mila caespitosa Britton & Rose	*Crozier DISS94*	Peru
Myrtillocactus geometrizans (Martius) Console	*Crozier DISS95*	Mexico
Neobuxbaumia mezcalensis (Bravo) Backeberg	*Crozier DISS96*	Mexico
Neolloydia conoidea (DC) Britton & Rose	*Crozier DISS97*	USA
Neoraimondia arequipensis (Meyen) Backeb.	*Crozier DISS98*	Peru
Obregonia denegrii Fric	*Crozier DISS99*	Mexico
Opuntia macrocentra Engelmann	*Crozier DISS100*	USA
Oreocereus celsianus (Salm-Dyck) Riccobono	*Crozier DISS101*	Peru
Oroya peruviana (Schumann) Britton & Rose	*Crozier DISS103*	Peru
Ortegocactus macdougallii Alexander	*Crozier DISS104*	Mexico
Pachycereus pringlei (Watson) Britton & Rose	*Crozier DISS105*	Mexico
Pachycereus schottii (Engelmann) D. R. Hunt	*Crozier DISS106*	Mexico
Parodia microsperma (Weber) Speg.	*Crozier DISS107*	Argentina
Parodia ottonis (Lehmann) Backeb.	*Crozier DISS108*	Argentina
Pediocactus simpsonii (Engelmann) Britton & Rose	*Crozier DISS109*	USA
Peniocereus greggii (Engelmann) Britton & Rose	*Crozier DISS112*	Mexico
Peniocereus striatus (Brandegee) Buxbaum	*Crozier DISS113*	Mexico
Pereskia aculeata Miller	*Crozier DISS114*	Mexico
Pereskia bahiensis Guerke	*Crozier DISS115*	Brazil
Pereskia bleo (Kunth) DC	*Crozier DISS116*	Panama
Pereskia diaz-romeroana Cárdenas	*Crozier DISS116*	Bolivia
Pereskia grandifolia Haworth	*Crozier DISS117*	Brazil
Pereskia guamacho Weber	*Crozier DISS118*	Venezuela
Pereskia horrida (Kunth) DC.	*Crozier DISS119*	Peru
Pereskia lychnidiflora DC.	*Crozier DISS120*	Mexico
Pereskia nemorosa Rojas	*Crozier DISS121*	Argentina
Pereskia portulacifolia (L.) Haw.	*Crozier DISS122*	Hispaniola
Pereskia quisqueyana Liogier	*Crozier DISS123*	Hispaniola
Pereskia sacharosa Griseb.	*Crozier DISS124*	Argentina

Pereskia stenantha Ritter	*Crozier DISS125*	Brazil
Pereskia weberiana Schumann	*Crozier DISS126*	Bolivia
Pereskia zinniiflora DC	*Crozier DISS127*	Hispaniola
Pereskiopsis porteri (Brandegee) Buxbaum	*Crozier DISS128*	Mexico
Pfeiffera ianthothele (Monville) Weber	*Crozier DISS129*	Bolivia
Phemeranthus calycinus (Engelm.) Kiger	*Crozier DISS130*	USA
Pilosocereus alensis (Weber ex Grosselin) Byles & Rowley	*Crozier DISS131*	Mexico
Portulaca sp	*Crozier DISS133*	USA
Portulacaria afra Jacq.	*Crozier DISS134*	South Africa
Pterocactus tuberosus (Pfeiffer) Britton & Rose	*Crozier DISS135*	Argentina
Quiabentia verticillata (Vaupel) Vaupel	*Crozier DISS136*	Argentina
Rebutia minuscula Schumann	*Crozier DISS137*	Argentina
Rhipsalis baccifera (J. S. Mueller) Stearn	*Crozier DISS138*	Mexico
Sclerocactus papyracanthus (Engelm.) N. P. Taylor	*Crozier DISS139*	USA
Sclerocactus polyancistrus (Engelm. & Bigelow) Britton & Rose	*Crozier DISS140*	USA
Sclerocactus scheeri (Salm-Dyck) N. P. Taylor	*Crozier DISS141*	USA
Selenicereus sp.	*Crozier DISS142*	Mexico
Stenocactus coptonogonus (Lemaire) Berger ex Hill	*Crozier DISS143*	Mexico
Stenocereus alamosensis (J. Coulter) Gibson & Horak	*Crozier DISS144*	Mexico
Stenocereus stellatus (Pfeiffer) Riccobono	*Crozier DISS145*	Mexico
Stenocereus thurberi (Engelmann) Buxbaum	*Crozier DISS146*	Mexico
Stephanocereus leucostele (Guerke) Berger	*Crozier DISS147*	Brazil
Stetsonia coryne (Foerster) Britton & Rose	*Crozier DISS148*	Argentina
Talinella pachypoda U. Eggli	*Crozier DISS150*	Madagascar
Talinum paniculatum (Jacq.) Gaertn.	*Crozier DISS151*	Peru
Tephrocactus inermis (Speg.) Backeb.	*Crozier DISS152*	Argentina
Thelocactus hexaedrophorus (Lemaire) Britton & Rose	*Crozier DISS153*	Mexico
Thelocactus setispinus (Engelmann) Anderson	*Crozier DISS154*	Mexico
Turbinicarpus saueri (Boedeker) John & Riha	*Crozier DISS155*	Mexico
Turbinicarpus schmiedickeanus (Boedeker) Buxbaum & Backeberg	*Crozier DISS156*	Mexico
Uebelmannia gummifera (Backeberg & Voll) Buining	*Crozier DISS157*	Brazil
Weberbauerocereus weberbaueri (Schumann ex Vaupel) Backeberg	*Crozier DISS158*	Peru
Yavia cryptocarpa Kiesling & Piltz	*Crozier DISS159*	Argentina

Six genera of the Portulacaceae and one genus each from families Basellaceae, Halophytaceae and Didiereaceae were chosen as outgroups based on the close relationship indicated by morphological studies (Thorne, 1983) and ribosomal DNA studies (Hershkovitz, 1991). These families were placed in the Portulacinae by Thorne (Cronquist & Thorne, 1994) and their monophyly has been supported by parsimony analysis of *ndh*F (Applequist & Wallace, 2001). However, the relationships between Portulacinae remain uncertain (Angiosperm Phylogeny Group, 2005).

To infer a plastid phylogeny for the Cactaceae three functionally diverse protein-coding regions were sampled: the *rbc*L gene encoding the large subunit of the essential photosynthetic protein complex RuBisCo; the *rpo*B gene encoding the RNA polymerase β subunit[7]; and the *mat*K gene that is unique as the only remaining gene in the chloroplast genome to code for a maturase. *Mat*K is located within the split intron of the *trn*K gene and the two genes are analyzed together with the intron in most studies. The second exon of *trn*K (only 35 base pairs) is included here in the phylogenetic analyses. All genes are located in the large single copy region of the chloroplast genome and all have been used widely in systematic studies, in the case of *rbc*L, since the earliest plant phylogenetic studies. Together these represent more than 6kb of nucleotide sequence, with *rpo*B representing about half that length.

DNA purification and sequencing

Total DNA was extracted from stem or leaf tissue of living or herbarium specimens using the organelle pellet method of Scott & Playford (1996). Contrary to popular myth, the Cactaceae presented no exceptional barriers to DNA extraction, although an additional phenol cleaning step was used for extremely mucilaginous

[7] The *rpo* gene family is highly variable (Goremykin ert al., 1997). *Rpo* genes encode core subunits of RNA polymerases that transcribe most photosynthesis-related genes (Suguira, 1992). However, promoter-specificity factors for the enzyme appear to be encoded in the nuclear genome (Hanaoka et al, 2003).

samples such as our samples of *Peniocereus* and most opuntioids (allies of *Opuntia*). As little as 0.19 g tissue provided enough DNA for amplification of all markers as well as experimentation, an important consideration when receiving small samples of rare or governmentally regulated species. DNA was amplified by PCR, purified, and sequenced as described in Panero & Crozier (2003) for Asteraceae samples. Several primers were designed to amplify the *rpo*B-*rpo*C genes that are co-transcribed and are listed in Table 2.2. All sequences were produced *de novo* for this study at the DNA core facility or Hillis-Bull Lab at the University of Texas at Austin using standard methods as referred to in Panero & Crozier (2003).

Table 2.2. Sequences of primers used for DNA amplification.

Markers	5' -3' primer sequence	Reference
***rpo*B-*rpo*C genes**	**5' -3' primer sequence**	**Reference**
rpoC1X1 47R	GAA ACT GAT CCA ATT CGG AG	This study
rpoB 3197R	TTA ATC TGG AAG TTC CTC TCA G	This study
rpoB 1977F	TAT GCC GTG GGA AGG TTA	This study
rpoB 2005F	GAT GCG GTA CTT ATT AGT GAG	This study
rpoB 2111R	TTT TCA GGA CCT TGA CTT GTC	This study
rpoB 865F	GCT GCG GAT CAT TTG ATT G	This study
rpoB 988R	CTA GAG CCA ATC CGA ATT G	This study
rpoB 3F	GCT ACG GGA TGG AAA TGA G	This study
rpoCR	GAA ACT GAT CCA ATT CGG AG	This study
rpoC2	CCC AAG CAC TTA TTT GTT GAG	This study
rpoB3Fspin	GCT ACG GGA TGG AAA TGA G	This study
rpo1583Rtob	TTT TCA GGG CCT TGG CTT GTC	This study
rpoB1583RSp	TTT TCA GGA CCT TGA CTT GTC	This study
rpo1679Ftob	TAT CGG GTG GGA GGG TTA C	This study
rpoB1679Fsp	TAT GCC GTG GGA AGG TTA C	This study
rpoB2704Rtob	CCA GAG CCA ATC CGA ATT G	This study

rpoB2791FTob	CTA GAG CCA ATC CGA ATT G	This study
rpoB2704RSp	GCT GCC GAT CAT TTG ATT G	This study
rpoB2791FSpi	GCT GCG GAT CAT TTG ATT G	This study
rpoBF tobacco	GCT CGG GGA TGG AAA TGA G	This study
rpoB-C2	CCC AAG CAC TTA TTT GTT GAG	This study
rpoB-CendR	TTA ATC TGG AAG TTC CTC TCA G	This study
1679Fsubst2	GAT GCG GTA CTT AGT GAG C	This study
***mat*K**		
trnK 3914F	TGG GTT GCT AAC TCA ATG G	Johnson & Soltis 1994
trnK2R	CTA CTC CAT CCG ACT AGT T	Johnson & Soltis 1994
matK-982R	TGA GTC TGT TGA TAC ATT CGG	This study
matK-905F	GAA AAT GCA GGC GAC AAG	This study
psbA-R	CGC GTC TCT CTA AAA TTG CAG TCA T	Johnson & Soltis 1994
matK982R	TGA GTC TGT TGA TAC ATT CGG	This study
matK905F	GAA AAT GCA GGC GAC AAG	This study
matK-4R	GCC AAA GTT CTA GCA CAA G	This study
matK8F	CTT CGA CTT TCT TGT GCT	Steele & Vilgays 1994
***rbc*L**		
rbcLR	GAT TTC CTT CCA TAC CTC AC	Panero & Crozier 2003
rbcL650	CAG GTG AAA TCA AAG GGC	Panero & Crozier 2003
rbcL1	ATG TCA CCA CAA ACA GAR ACT AAA GC	Olmstead, 1992
rbcL2	CTT TTA GTA AAA GAT TGG GCC GAG	Olmstead, 1992
rbcL2Rint	TCC ACC AGA CAG ACG TAA CG	This study

Phylogenetic analysis

Alignment

Raw nucleotide sequences were proofread and trimmed using Sequencher (v3.1 or 4.1, Gene Codes Corp.) and the assembled 'contig' matrices for each sequencing primer interleaved into genomic units using PAUP* (v4.0b10, Swofford, 2000). Pseudogenes,

discovered by the presence of internal stop codons or other anomalies, were discarded and the sample re-amplified and sequenced. Although not trivial, correct assessment of nucleotide homology by alignment of sequence data is generally more straightforward for coding than non-coding sequence data, and the insertion of very few gap regions was required for these data sets. Many studies report the use of popular multiple alignment software such as Clustal (Thompson *et al*., 1997) to hypothesize positional homology, even though the objectivity of computer-generated alignment is lost when assumptions are violated by manual adjustments "by eye" after alignment. Because sequence similarity was high translated codons were aligned manually using MacClade (v.4.07, Maddison & Maddison, 2000), then checked and adjusted to reflect observed nucleotide changes missed by the peptide alignment, for example small inversions, or (in a single case) a deletion of nucleotides not contiguous with triplet codons. The inverse relationship between taxon-sampling density and alignment ambiguity was clearly observed during this step. Gaps were treated as missing data in analyses.

Molecular character evolution statistics

For each gene differences in base composition among taxa were examined for all informative sites using the homogeneity χ^2 test implemented in PAUP*. Base compositional bias at sites across the sequence length were calculated and summarized in a histogram showing their relative frequencies using Mesquite (v 1.01, Maddison & Maddison, 2002-2004).

The incongruence length difference test (ILD; Farris et al., 1994) was implemented in PAUP* to explore possible incongruence between pairs of character sets, rather than as a strict test of data set combinability. Multiple pairwise tests were performed between each pair of loci, and between codon positions for each locus removing invariant characters first as suggested by Cunningham (1997). Several recent

studies e.g., Dolphin *et al*. (2000), Darlu & Lecointre (2002), and especially that of Barker & Luzoni (2002) make a strong case against the utility of this test as 1) a measure of phylogenetic congruence, 2) an indicator of lineage-specific or site specific heterogeneity, or 3) a criterion for data partition combinability. Although combining incongruent partitions can sometimes actually increase phylogenetic accuracy (Weins 1998, Cunningham 1997) the ILD apparently offers little power to predict the influence of combining data partitions on phylogenetic accuracy. Nonetheless this test has remained in use in plant phylogenetic studies and is included here.

Many phylogenetic methods assume rate constancy of nucleotide substitutions across lineages, so a likelihood-ratio test (Hasegawa, Kishino & Yano, 1985) was used to test the goodness-of-fit of these data to a model constrained to ultrametricity versus one of rates free to vary across the tree. This was implemented using the "enforce molecular clock" option in PAUP*. Significance of the -2logLikelihood test statistic was approximated using the χ^2 distribution at the 99% significance level. This global test of a molecular clock does not identify specific branches that may contribute to rate heterogeneity across the tree.

Assessment of rates across lineages

Relative rates of molecular evolution across lineages were investigated using the Bayesian test described in Wilcox et al. (2004). During the Bayesian tree search of the concatenated data set (see ***Phylogenetic Inference*** section below) the posterior probability distribution of branch lengths was saved. For each tree sampled after stationarity, distances from each terminal taxon to a node representing the most recent common ancestor (MRCA) of the Cactaceae and outgroups were summed using CADENCE (v1.0, Wilcox *et al.*, 2004). For each terminal taxon the distribution of distances obtained from all trees sampled after stationarity were plotted along with the

95% confidence intervals of those estimated distances. Significantly different evolutionary rates are inferred if confidence intervals on the means of two distributions do not overlap. This test has the advantage over other relative rates tests by taking tree structure into account and allowing multiple comparisons simultaneously without a loss in power.

Evolutionary model selection

Selection of best-fit models of nucleotide and amino acid substitution from among a set of standard models was accomplished in Modeltest 3.06 (Posada & Crandall, 1998) respectively and evaluated using the Akaike Information Criterion (AIC, Akaike, 1974). The gamma distributions for Bayesian analyses were approximated with six rate categories: two transition rate classes and four transversion rate classes.

Phylogenetic inference

Maximum parsimony (MP), maximum posterior probability (Bayesian), and minimum evolution (ME) methods were used to estimate topology for each locus individually and as a concatenated set. MP and ME tree searches were implemented in PAUP*. As an alternative to searching for an optimal (shortest) tree under a MP or ME criteria, Bayesian inference was used to sample possible trees according to their posterior probability calculated using Bayes' theorem. Bayesian analyses were implemented in MrBayes (v. 3.0b4 either serial or parallel versions, Huelsenbeck & Ronquist, 2001). All data partitions analyzed and discussed here are taxonomically equivalent.

Because of the relatively large number of taxa, MP heuristic searches to find most parsimonious trees were conducted as follows: First, 100 random taxon addition replicates were performed with only 10 trees saved each iteration. Trees saved by this search were then branch-swapped using tree bisection-reconnection (TBR) to check for

shorter solutions and to fill out tree space to a limit of 5000 trees at this length. Equally weighted characters were optimized by accelerated transformation (ACCTRAN). Bootstrap resampling (Felsenstein, 1985) with 100 pseudoreplicates was used to evaluate internal clade support.

Bayesian inference was accomplished via a Metropolis-coupled Markov-chain Monte Carlo (MCMCMC; Li et al, 1996; Larget & Simon, 1999; Huelsenbeck & Ronquist, 2001) approximation running four simultaneous MCMC chains: one cold and 3 incrementally "heated", temp=0.5, to facilitate mixing. Chains were run 4 million cycles for each single locus analyses and 10 million cycles for the concatenated data set. All priors were set according to the preferred GTR model stated above, dirichlet priors for the rate matrix, and uniform priors for the shape and proportion of invariant sites. All runs were started from random trees and log-likelihoods and trees including branch lengths were sampled every 100 generations. Four independent replicates of each analysis were run and compared for apparent stationarity levels to check that analyses had not been trapped in local optima. Log likelihood values of sampled trees were plotted against generation number and compared with graphs of replicate runs to determine the initial point at which stationarity was reached (not shown). Trees sampled before stationarity were discarded as burn-in. The mean, standard deviation and 95% confidence interval of model parameters was calculated for trees sampled after stationarity. Probabilities $\geq$95% are considered significant.

Minimum evolution trees were derived from log-determinant (log-Det) distances of variable sites evolution. In contrast to the GTR model, the log-Det distance transformation corrects for unequal base frequencies in each pairwise comparison rather than an average applied to all comparisons, and does not assume stationarity of rates across the tree (Lockhart *et al*. 1994; Steel, 1994; Steel, Huson & Lockhart, 2000).

Removing invariant sites in proportion to base composition at constant sites may alleviate the weakness of this method ie. accommodating rate variation across sites. Starting trees were calculated using the neighbor-joining algorithm.

Hypothesis testing

A parametric bootstrap test (Goldman *et al*. 2000; Hillis *et al*. 1996; Huelsenbeck *et al*. 1996) was performed to test the monophyly of *Pereskia*. A model tree consistent with the *a priori* hypotheses of a monophyletic *Pereskia* (Figure 2.1) was chosen from among the best trees resulting from the maximum parsimony analysis of the combined data and used to simulate 100 replicate data sets in Seq-Gen (v1.2.5, Rambaut & Grassly, 1997). The null distribution of differences in parsimony scores generated from the best trees found in unconstrained and searches constrained only to *Pereskia* monophyly for each of the replicate datasets was used to compare that of observed data and assessed at the 99% significance level. For other hypotheses based on tree topology the trees sampled after stationarity in Bayesian analysis were filtered with the appropriate constraint tree and the number of filtered trees divided by the total number of post-stationarity trees to obtain a posterior probability of the hypothesis represented in the constraint tree.

Figure 2.1. Model tree used to simulate data sets for null distribution.

RESULTS

Data characteristics

Sequence alignment resulted in 6,422 homologous nucleotide sites from each of the 122 taxa sampled. This total consisted of 1,575 sites from *mat*K, 35 sites from *trn*K exon2, 1,476 sites from *rbc*L and 3,336 sites from *rpo*B. Of the total 1,931 were variable and 1,002 represent parsimony informative characters (Table 2.3). Due to the location of the *rbc*L1 primer, many of the first 50 base pairs at the start of most *rbc*L sequences were essentially unreadable and these sites were omitted from analysis. In total 414 sites were deemed ambiguously aligned or contained missing or ambiguous data and were omitted from the analysis.

Table 2.3. Summary of base composition and nucleotide substitution process statistics.

Partition	# sites	# sites analysed	variable sites	MP info sites	Chi^2 Test of Base Frequency Homogeneity (df=363)	Chi^2 Test of Molecular Clock	% *Invariant*	α	MODELTEST		ProtTest		
									hLRT	AIC	lnL	AIC	BIC
matK	1575	1527	568	278	X^2 = 21.020230 P = 1.00000000	P<<0.001	0*	0.6485	TVM+ I+Γ	TVM+ I+Γ	JTT+Γ+F	JTT+Γ+F	JTT+Γ+F
trnK exon 2	35	35	9	2	X^2 = 14.191512 P = 1.00000000		0*	0.6198	JC	TVM			
rbcL	1476	1320	321	177	X^2 = 11.143335 P = 1.00000000	P<<0.001	0.5024	0.9168	TIM+ I+Γ	GTR+ I+Γ	JTT+Γ+F	JTT+Γ	JTT+Γ
rpoB	3336	3156	1043	547	X^2 = 23.712553 P = 1.00000000	P<<0.001	0.3625	0.8229	GTR+ I+Γ	GTR+ I+Γ	JTT+ I+Γ+F	JTT+ I+Γ+F	JTT+ I+Γ
GENES	***6422***	***6008***	***1931***	***1002***			0.3867	0.9078	GTR+ I+Γ	GTR+ I+Γ			

Within each locus homogeneity of base frequencies was inferred based on results of the chi-squared test (Table 2.3). During the hierarchical model selection procedure

implemented in MODELTEST (v3.06, Posada & Crandall, 1998) the shape parameter (α) was estimated to be <1.0 indicating monotonically decreasing discrete gamma distributions for each locus, and values for the proportion of invariant sites ranged from zero in *mat*K-*trn*K to more than half in *rbc*L (Table 2.3). Therefore, the base-substitution process was found to be heterogeneous among process partitions of the data. The General Time Reversible model (GTR: Lanave *et al.*, 1984; Tavare, 1986; Rodriguez *et al.*, 1990) with discrete gamma distributed rate variation across sites (Γ; Yang, 1993) and correction for proportion of invariant sites (I; Gu *et al*, 1995; Waddell & Penny 1996) was preferred for all partitions as well as for the combined data set (Table 2.3). Since the best-fitting model for *mat*K, the transversion model with gamma distribution and proportion of invariant sites (TVM+I+Γ) is not implemented in MrBayes. The GTR + pINV + Γ was the most highly parameterized model tested.

As expected for chloroplast DNA all three genes have an A-T base compositional bias. Averages of G-C content varied across loci from a high 43.26% for *rbc*L, 38.49 for *rpo*B and low 32.21% for *mat*K-*trn*Kexon2 (Figure 2.2).

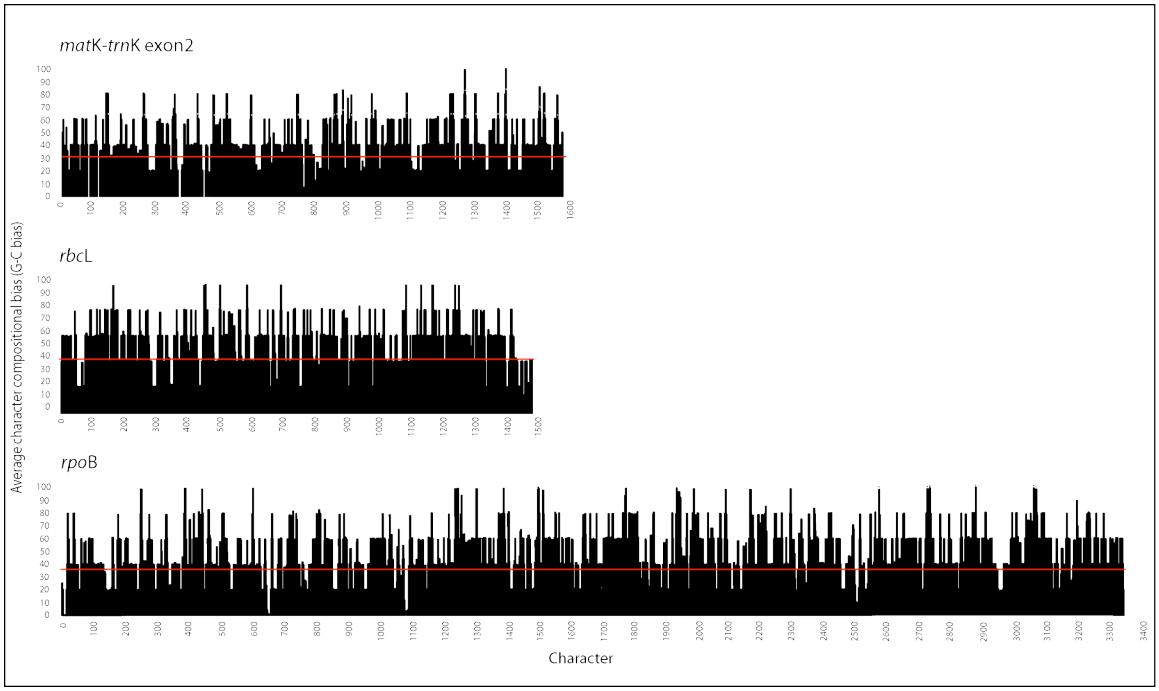

Figure 2.2. Average character compositional bias (G-C bias) along gene sequence for all taxa. Horizontal bar (red) indicates average for the gene: *mat*K-*trn*Kexon2 = 32.21, *rbc*L = 43.26, *rpo*B = 38.49.

Partition heterogeneity

Phylogenies reconstructed from different loci are expected to be congruent except in minor parts of their histories because chloroplast genes are physically linked and recombination is minimal if it exists (Weins, 1998; de Queiroz, 2002). However, the ILD test used to assess topological congruence (partition homogeneity) indicated that *mat*K, *rbc*L and *rpo*B should be considered incongruent partitions of the data at the 95% confidence level. At the same time, the ILD test could not reject congruence when data

were partitioned by first, second and third codon positions (Figure 2.3). In other words, the ILD tests show more conflict between loci than between codon positions.

a.

matK vs rbcL	0.01*
matK vs rpoB	0.02*
rbcL vs rpoB	0.01*

b.

	matK	rbcL	rpoB
1st vs 2nd	0.50	0.67	0.44
1st vs 3rd	0.75	0.10	0.58
2nd vs 3rd	0.72	0.49	0.60
1st+2nd vs 3rd	0.68	0.15	0.88

*significant to reject homogeneity

Figure 2.3. Results of the Incongruence Length Difference (ILD) Test. Significant process partition heterogeneity was observed (a.), whereas codon position homogeneity (b.) was indicated by partition homogeneity tests implemented in PAUP*.

Each locus failed a global test of the molecular clock with p-values much less than 0.001.

Relative rates among lineages

Distributions of branch lengths were significantly different across the Cactaceae tree. Surprisingly, two groups of *Pereskia* species had non-overlapping 95% confidence intervals. Five pereskias, including the widespread *P. aculeata*, Mexican *P. lychnidiflora* and three Andean pereskias had distributions overlapping with members of the Opuntioideae. The confidence intervals of the remaining ten species of *Pereskia* overlap

neither the Andean *Pereskia* clade nor the Opuntioideae. Distributions for *Maihuenia* overlapped those of both *Pereskia* groups. Branch lengths to *Blossfeldia* and basal taxa of Rhipsalidoideae and Cactoideae were similar to and overlap those of Opuntioideae. The recently derived *Mammillaria* however, showed another increase in relative rate (Figure 2.5).

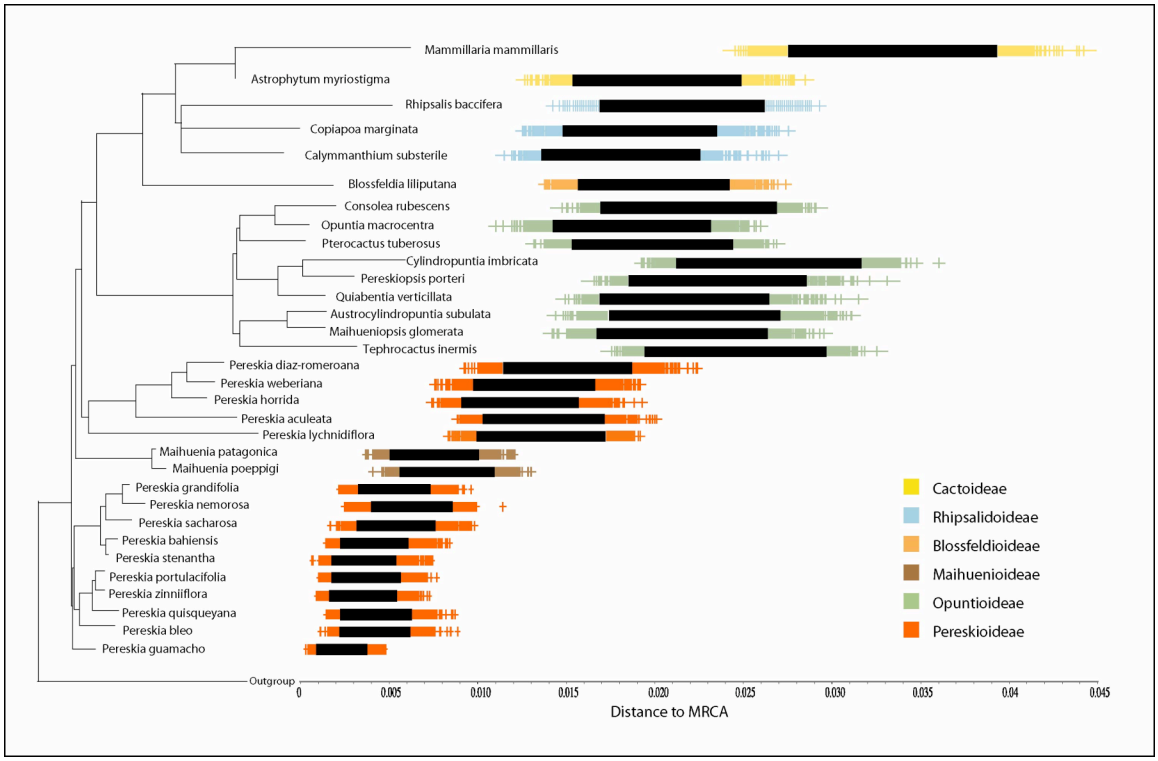

Figure 2.5. Rate variation among major lineages of Cactaceae. Distributions of branch lengths from most common recent ancestor (MRCA) to each terminal taxon on trees resulting from combined analysis of genes assuming GTR+pINV+Γ model. Bayesian 95% confidence intervals are shown in black. Taxon labels to the left of each distribution are shown on truncated minimum evolution tree.

Tree topologies, branch support and probabilities of splits

Trees resulting from each maximum parsimony search are summarized by the strict consensus topology of trees. In Bayesian analyses of individual loci, log-likelihood values converged on similar mean values (plateau) after approximately 200,000 generations in each analysis except that of the *mat*K gene that converged near 600,000 generations. The first 2,000 and 6,000 (*mat*K) trees were discarded as burn-in (Huelsenbeck & Ronquist, 2001). The majority rule consensus trees with posterior probabilities were determined from 38,000 trees except in the case of *mat*K (34,000 trees).

Phylogenetic analyses by different methods and of different partitions consistently found strong support for several major clades including the Cactoideae, Rhipsalidoideae, Blossfeldioideae and Opuntioideae, and two unnamed clades within the Rhipsalidoideae that encompass traditional tribes as follows: clade A. Pachycereeae, Hylocereeae, Leptocereeae and *Neoraimondia*, and clade B. Trichocereeae, Cereeae, Browningieae, Notocacteae, Rhipsalideae (Figures 2.6, 2.7, 2.8).

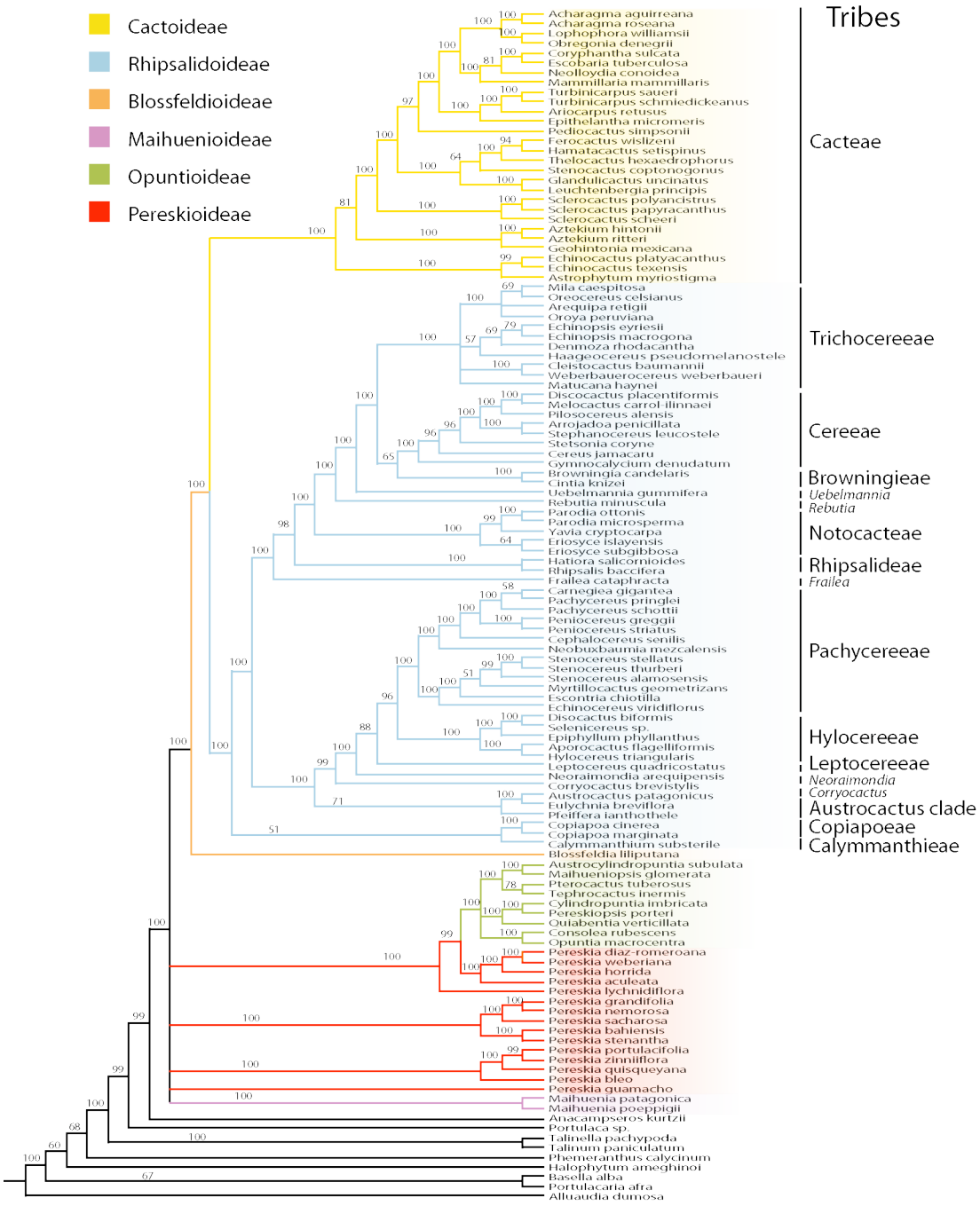

Figure 2.6. Majority rule tree. Consensus of 98,000 post-burn-in trees sampled by Bayesian MCMC analysis of combined *mat*K, *rbc*L and *rpo*B data. Posterior probabilities of clades shown above the branches.

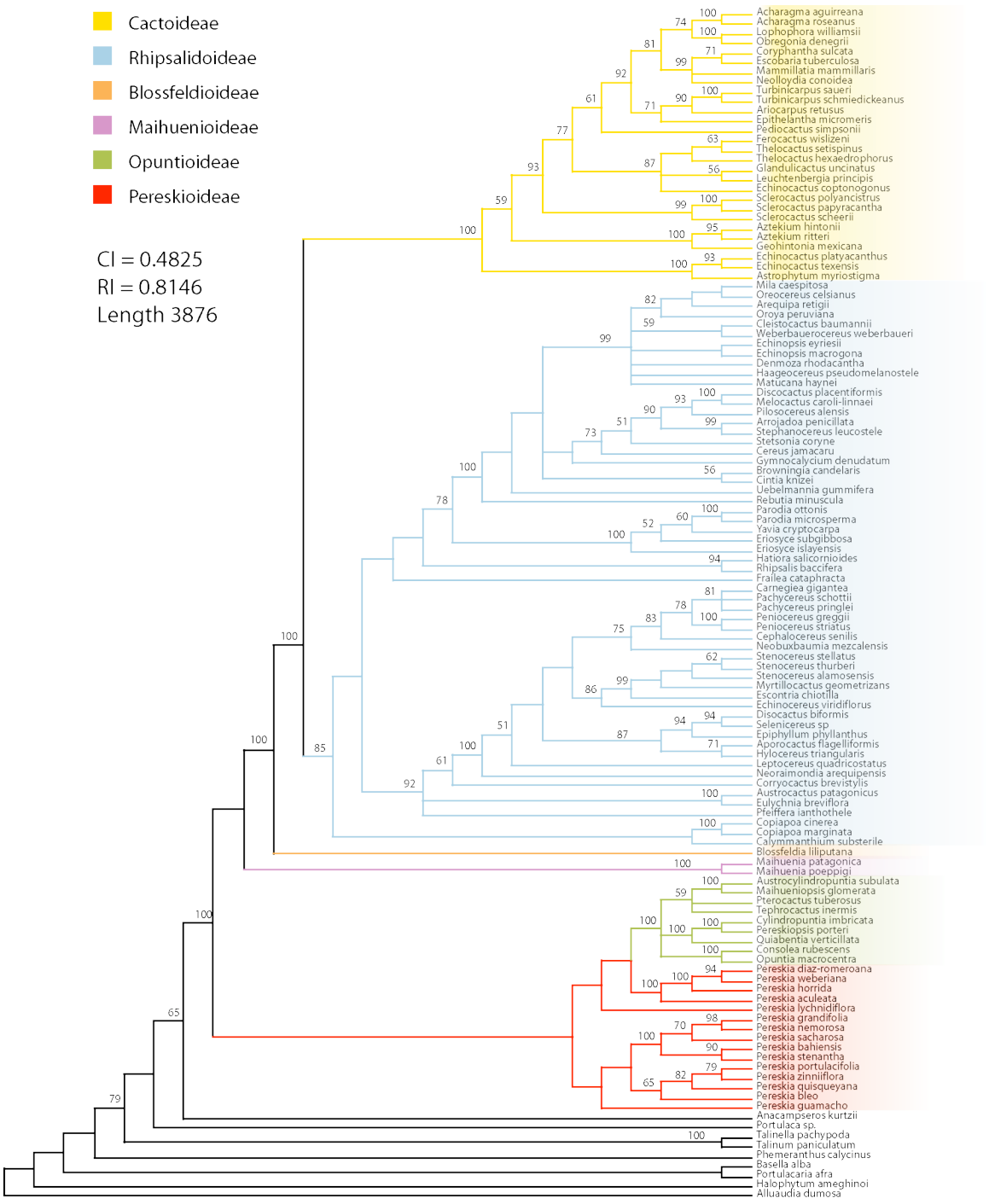

Figure 2.7. Strict consensus tree. Strict consensus summarizing 2009 most parsimonious trees found in unweighted maximum parsimony heuristic search of the combined three-gene data set.

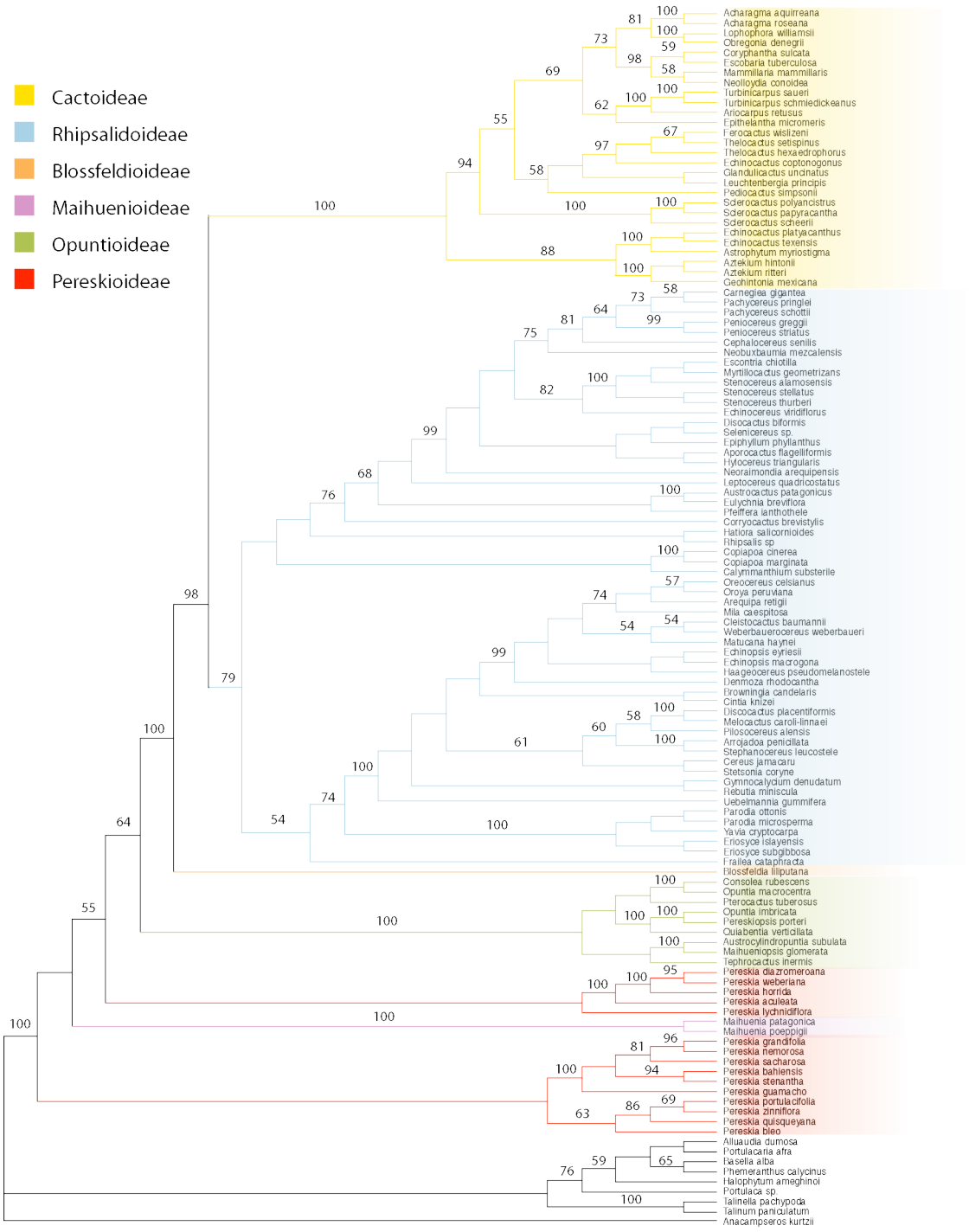

Figure 2.8. Minimum evolution tree using pinv-logDet model to correct distances for non-stationarity across the tree. Bootstrap values shown above branches.

In single-locus analyses only those of the *rpo*B partition joined pereskias with Opuntioideae (Figures 2.9, 2.10, 2.11). In Bayesian and minimum evolution analyses of *rbc*L pereskias and *Maihuenia* form a weak clade (53% posterior probability); *Pereskia* clades and species with *Maihuenia* are unresolved at the base of the tree under parsimony criterion. Neither Bayesian nor minimum evolution analysis of *mat*K resolved *Pereskia* clades and species, and *Maihuenia,* with Opuntioideae (Figures 2.9, 2.11). Parsimony analysis of *rbc*L joined Opuntioideae with the Blossfeldioideae, Rhipsalidoideae and Cactoideae in the strict consensus but without bootstrap support >50% (Figure 2.10). In contrast, analyses of *rpo*B join the Andean *Pereskia* clade, *P. lychnidiflora,* and southeastern S.A. *Pereskia* clade with Opuntioideae with 82% posterior probability in Bayesian analysis. *Blossfeldia* was found to be an independent lineage sister to the rest of the traditional Cactoideae in all analyses. *Blossfeldia* shares with the Opuntioideae, pereskias and *Maihuenia* numerous molecular synapomorphies. *Blossfeldia* does not share the loss of the *rpo*C1 intron, recognized as a molecular synapomorphy for clade containing all other cactoid cacti (Wallace & Cota, 1995; Nyfeller, 2002; Angiosperm Phylogeny Group, 2005) nor deletions in the *trn*T-*trn*L intergenic spacer deemed informative for identifying groups within Cactoideae-Rhipsalidoideae by Applequist & Wallace (2002).

Figure 2.9. Majority Rule consensus of 38,000 (34,000 *mat*K) post burn-in trees sampled by Bayesian MCMC for each locus: *mat*K, *rbc*L, and *rpo*B. Posterior probabilities of clades shown above the branches.

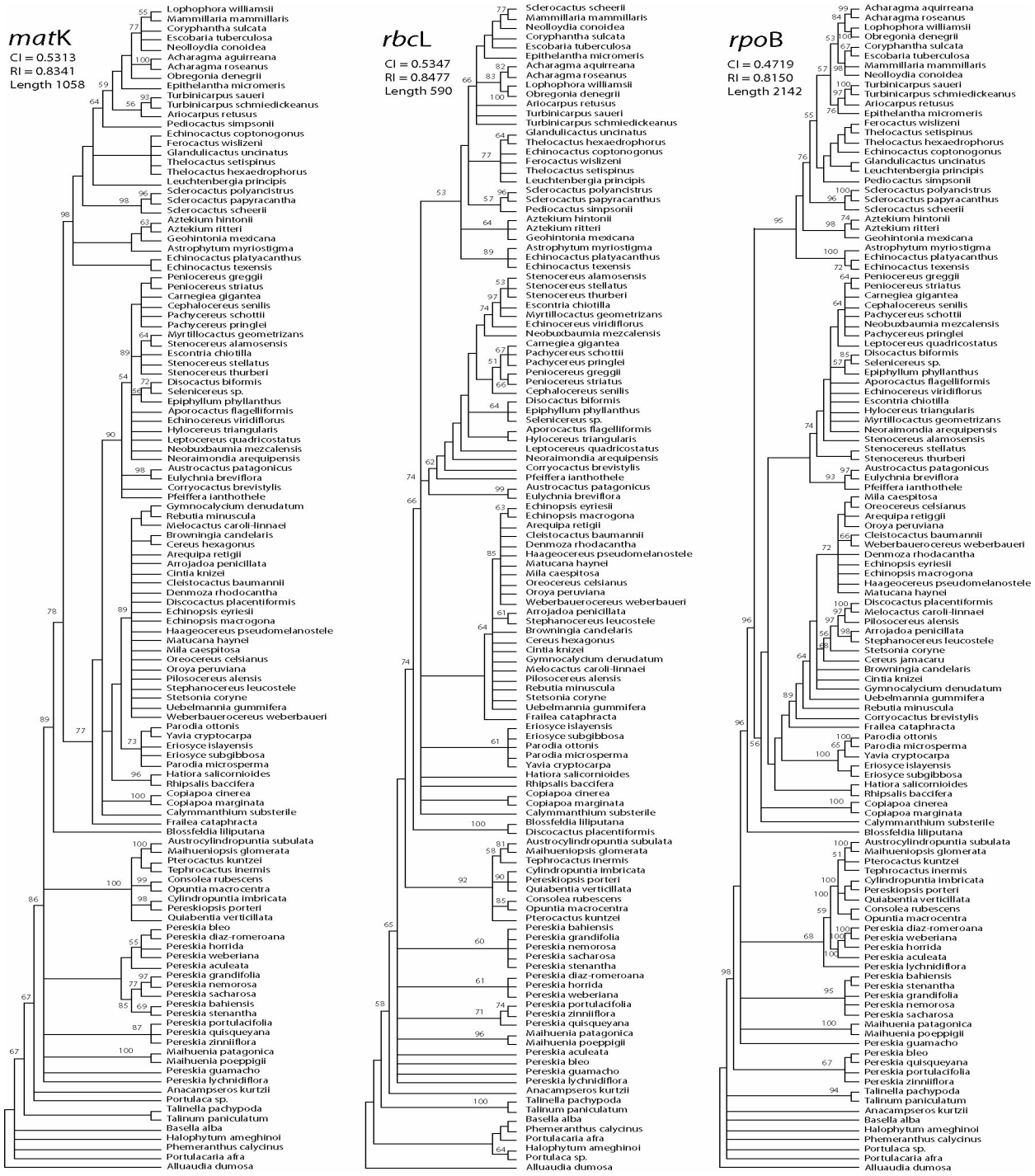

Figure 2.10. Strict consensus of 5,000 most parsimonious trees found in unweighted maximum parsimony heuristic searches for each locus: *mat*K, *rbc*L, and *rpo*B. Bootstrap proportions shown above branches.

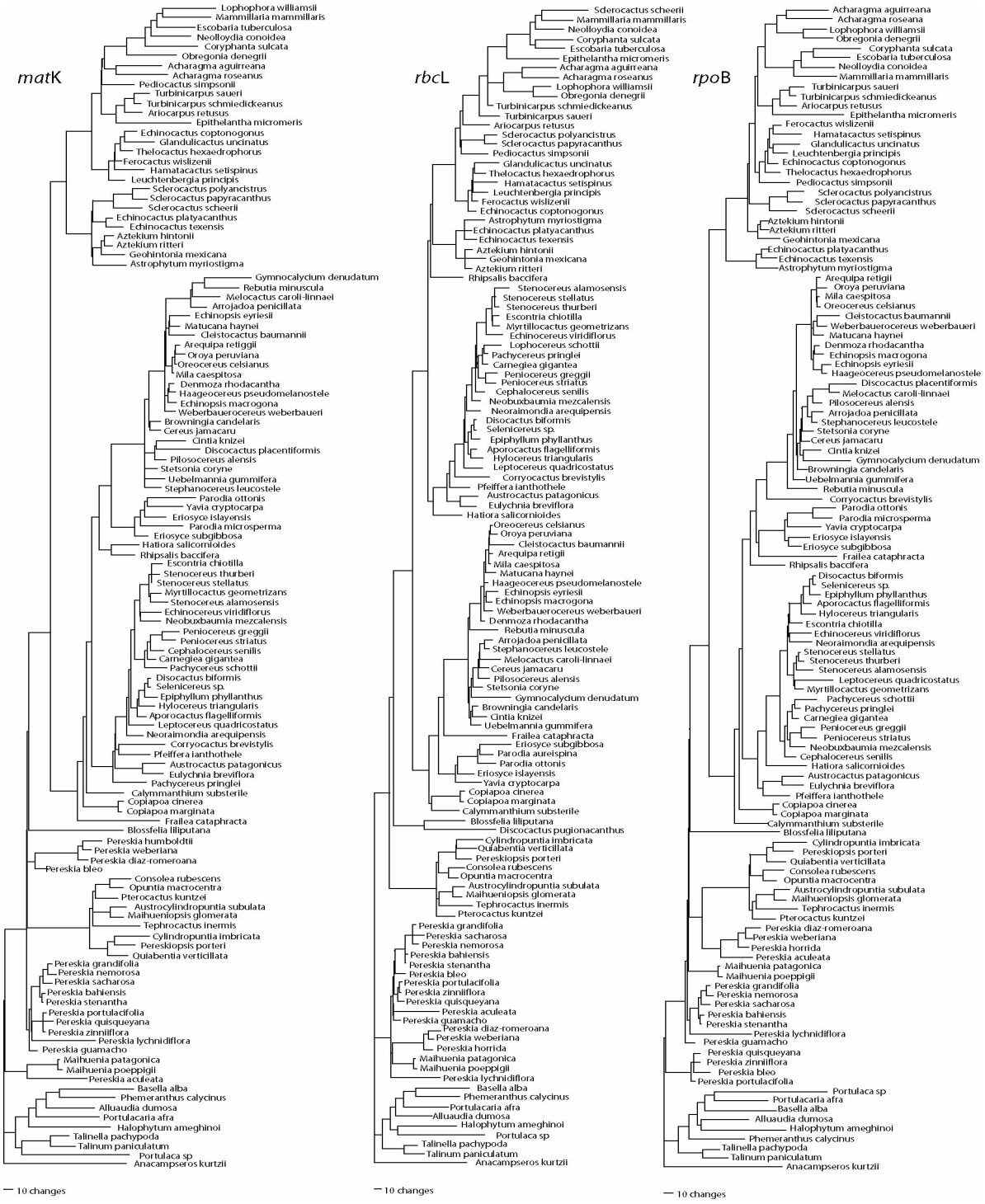

Figure 2.11. Phylograms from minimum evolution trees found using pinv-logDet model to correct distances for non-stationarity across the trees of *mat*K, *rbc*L, and *rpo*B.

Combined analyses, both parsimony and Bayesian, as well as separate Bayesian analyses of *mat*K and *rpo*B resulted in identical relationships within the Cactoideae with the exception that the *Neolloydia* clade collapsed to a polytomy with *Mammillaria* in the combined parsimony and *rpo*B parsimony topologies (Figure 2.6, 2.7). The minimum evolution tree (Figure 2.8) shares most of the same relationships with two exceptions: 1. *Echinocactus+Astrophytum* is sister to *Aztekium+Geohintonia* rather than basal in the subfamily, and 2. *Pediocactus* is sister to the *Leuchtenbergia* clade rather than sister to the *Leuchtenbergia* clade + Coryphanthinae + *Ariocarpus* clade. The subfamily is divided into seven clades plus the *Pediocactus* lineage strongly supported by Bayesian posterior probabilities or moderately to strongly supported clades in a fast parsimony bootstrap.

In all combined analyses the Rhipsalidoideae contains two large clades (Figures 2.6, 2.7, 2.8). One clade comprises tribes Pachycereeae, Hylocereeae, Echinocereeae, and Leptocereeae along with 5 genera outside the clades of these tribes: *Neoraimondia*, *Corryocactus*, *Austrocactus*, *Eulychnia* and *Pfeiffera*. This clade is supported by 100% posterior probability and 92% bootstrap values. The other large clade comprising tribes Browningieae, Cereeae, Trichocereae and Notocacteae, is supported by 100% posterior probability but only a 78% bootstrap value. *Rhipsalis*, *Hatiora* and *Frailea* are joined to this clade in Bayesian topology but collapse to a polytomy in the strict consensus parsimony tree. None of the separate analyses of *mat*K and *rbc*L resolved the placement of *Rhipsalis*, *Hatiora* or *Frailea* (Figures 2.9, 2.10, 2.11). Minimum evolution analyses of *rpo*B and *rbc*L break *Rhipsalis* and *Hatiora* sister pair and places them into different clades. (The sister relationship of these two species is expected based on morphological evidence.). Both the large clades of Rhipsalidoideae are strongly supported by *mat*K and moderately supported by *rpo*B in Bayesian and parsimony analyses analysis. If

Notocacteae is excepted then both clades are strongly supported by the *rbc*L partition as well. A third element of Rhipsalidoideae concerns *Copiapoa* and the monotypic *Calymanthium*. All analyses resulted in inclusion of these taxa in the Rhipsalidoideae clade, either placing them in a polytomy with the two large clades or sister to the pair. The combined Bayesian analysis (Figure 2.6) places them sister to the two large clades with 100% support but clusters them with only 51% posterior probability. In all separate analyses *Copiapoa* and *Calymanthium* collapse unresolved outside these two clades (Figures 2.9, 2.10, 2.11).

Blossfeldia is very strongly supported as sister to the Cactoideae-Rhipsalidoideae phylad in all combined and individual locus analyses (99% bootstrap values and 100% posterior probabilities). No other members of this clade were observed in any analyses except that *Discocactus* was found sister to *Blossfeldia* in individual analyses of the *rbc*L partition by all methods (Figures 2.9, 2,10, 2.11).

The monophyly of the Cactaceae is well supported by my data, congruent with morphological studies (Gibson & Nobel, 1986) and previous molecular studies (Hershkovitz & Zimmer, 1997; Nyffeler, 2002), and strongly supported with 100% bootstrap and Bayesian probability. My data also show that the Portulacaceae is apparently not monophyletic but rather a series of sequentially splitting lineages leading to the Cactaceae. This result is not unusual and has been reported extensively in the literature (see Stevens, 2004 for references and commentary). Results of Bayesian and parsimony analyses support the recognition of at least 6 subfamilies in the Cactaceae. Relationships among the basal lineages of Cactaceae were not unequivocally resolved by parsimony or Bayesian methods. The Opuntioideae clade was supported by 100% bootstrap and posterior probabilities values in all analyses, but the *Maihuenia* clade, and three clades of pereskias, plus the yellow-flowered *P. lychnidiflora* and *P. guamacho*,

appear variously placed in analyses by different methods. In the 3-gene combined analyses the Andean pereskias (*P. diaz-romeroana*, *P. weberiana* and *P. horrida*) with *P. aculeata* formed a strongly supported clade; the southeastern South American *Pereskia* species (*P. grandifolia*, *P. nemorosa*, *P. sacharosa*, *P. bahiensis* and *P. stenantha)* formed a strongly supported clade, and the Caribbean *Pereskia* species (*P. portulacifolia*, *P. zinniiflora*, *P. quisqueyana* and *P. bleo*) formed a strongly supported clade. In the Bayesian analysis the Andean *Pereskia* clade along with *P. lychnidiflora* formed a clade with Opuntioideae supported by 100% posterior probability (Figure 2.6). However, in the combined parsimony analysis Opuntioideae, the three *Pereskia* clades, the *Maihuenia* clade, *P. lychnidiflora* and *P. guamacho* formed a basal polytomy (Figure 2.7). Under the $_{p}$inv log-Det transformed minimum evolution criterion two clades of pereskias and a *Maihuenia* clade formed a basal grade to the Opuntioideae with the *Maihuenia* clade a step between the *Pereskia* clades (Figure 2.8).

Relationships between the basal taxa, Opuntioideae, *Maihuenia* and multiple clades of *Pereskia* varied across analyses. The a priori hypothesis of a monophyletic *Pereskia* was rejected by the parametric bootstrap analysis (Fig. 2.12).

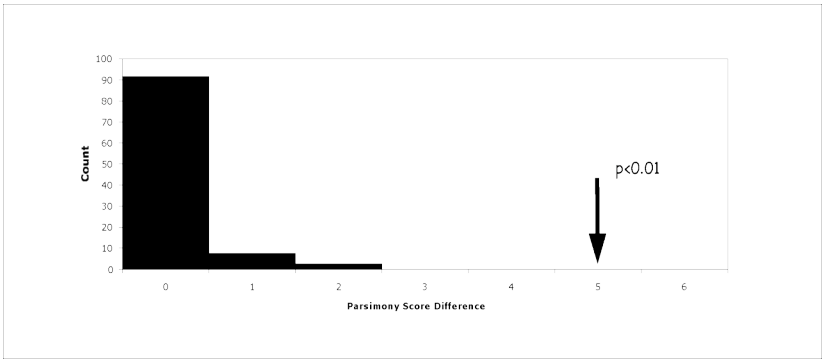

Figure 2.12. Results of parametric bootstrap test of null hypothesis of *Pereskia* monophyletic.

DISCUSSION OF CACTACEAE SYSTEMATICS

Phylogenetic relationships

Congruent results from independent loci of the chloroplast provide strong evidence of major clades in Cactaceae and their relationships, altering the earlier view of Cactaceae as evolving along only three morphologically distinct lineages. These results have precipitated a revision of Cactaceae classification (Chapter 4, 5) that recognizes six subfamilies (Crozier, 2004): Pereskioideae, Maihuenioideae, Opuntioideae, Blossfeldioideae, Cactoideae, and Rhipsalidoideae.

Surprisingly, the enigmatic monotype *Blossfeldia* was found to be a link between the basal lineages Opuntioideae-*Pereskia-Maihuenia* and Cactoideae-Rhipsalidoideae sharing many molecular synapomorphies[8] with the former. Morphological studies have consistently placed this taxon squarely within the cactoid group in tribe Notocacteae, never before suggesting it could provide a link between the cactoid cacti and the opuntioids and pereskioids[9]. The taxonomic implications resulting from the phylogenetic position and distinctiveness of this lineage are discussed in detail in Chapter 5.

A strongly supported dichotomy places the North American dwarf cactus clade Cactoideae as sister to a large clade including tribes Pachycereeae, Hylocereeae, Notocacteae, Rhipsalideeae, Trichocereeae, Browningieae, Leptocereeae, Cereeae and Calymanthieae along with *Copiapoa*. This clade had not been recognized previously in morphological analyses. Based on analysis of *trn*K/*mat*K and the *trn*L-*trn*F intron

[8] Including the *rpo*C1 intron, sampled as part of the study in Chapter 3, and considered by many to be a synapomorphy for the traditional Cactoideae.

[9] This result was found concurrently in a blind study (Nyffeler, 2002) using the two of the same markers used in this dissertation study. This result was so unexpected that Dr. Nyffeler requested, and was sent, the *Blossfeldia* trnK/matK sequence from this study for comparison with his results prior to his publication.

Nyffeler (2002) also identified the large clade with 90% bootstrap support, but misidentified the group *as* the "core Cactoideae" when in fact the clade *is sister to* the core of Cactoideae containing the type species (see Chapter 5). Instead, Rhipsalidoideae is nomenclaturally appropriate and used here. Nyfeller did not note the evolutionary significance on this sister relationship; the Cactoideae is actually a much older lineage than previously thought. Prior to molecular analyses the Cactoideae (=Cacteae) had been thought to have been derived from somewhere within the Rhipsalidoideae, with most cases speculating the Notocacteae or Pachycereeae (Zimmermann, 1985). Cactoideae is restricted to North America with the exception of one or two species along the northern coast of South America belonging to the derived genus *Mammillaria*. Without any other geographical connection to the basal taxa in the sister clade Rhipsalidoide (e.g., *Copiapoa, Calymanthium*) in South America, its origin predating the Isthmus of Panama[10] and therefore the Cactoideae is likely the result of long distance dispersal as Simpson & Neff (1985) have described for other plant disjunctions. Further, this is one of the few examples of a successful radiation into the deserts of Mexico that is of South American origin.

Within Rhipsalidoideae two distinctive genera, *Copiapoa* and the monotypic *Calymanthium*, are found to be sister to a large unnamed clade comprising Rhipsalideae, Notocacteae, Leptocereeae, Hylocereeae, Pachycereeae, Cereeae, Browningieae and Trichocereeae. Two sister clades comprise these eight tribes; their strongly supported

[10] Evolutionary divergence times in Cactaceae were calculated using the penalized likelihood method implemented in the program r8s (v 1.50; Sanderson, 2002b) for the most parsimonious tree resulting from a MP analysis with reduced taxon sampling (results not shown). In the absence of Cactaceae macrofossils calibration was accomplished by first estimating the divergence of Cactaceae on a tree inferred from combined analysis of atpB, rbcL and 18S sequence data downloaded from Genbank for 22 Caryophylales, (incl. *Pereskia aculeata*) and 8 outgroup families, fixing the divergence dates of Caryophyllalles, Dilleniaceae, and Polygonalles (Magallón & Sanderson, 2001) and Aizoaceae (Klak, Reeves & Hedderson, 2003). Standard errors for divergence times were calculated via simulation using Seq-Gen (v1.2.5, Rambaut & Grassly, 1997).

sister relationship has no precedent in either morphological or molecular studies. The nominal clade containing Rhipsalideae includes also Notocacteae, Browningieae, Cereeae, and Trichocereae along with *Frailea*, *Uebelmannia* and *Rebutia* that are each identified here as distinct lineages. However, the placement of Browningieae is only marginally supported by low posterior probability in the Bayesian combined analysis and this signal was contributed primarily by the *rpo*B data. The sister clade comprises a grade from the independent lineages *Corryocactus* and *Neoraimondia* to Leptocereeae, Hylocereeae, Pachycereeae, sister to the clade comprising *Pfeiffera*, *Eulychnia* and *Austrocactus* (unnamed). The derived tribe Pachycereeae comprises the North American columnar cacti. The Pachycereeae contains two sister clades currently recognized as subtribes Pachycereinae and Stenocereinae. The sister taxon relationship is also supported by recent non-coding chloroplast DNA studies (Arias-Montes & Terrazas, 2003). Distinct clades of epiphytes, Rhipsalideae and Hylocereeae respectively, have arisen in parallel in each of these clades. Likewise, columnar and globular forms have evolved in parallel.

Relationships among basal lineages

Basal relationships in Cactaceae between Opuntioideae (300+ species), *Maihuenia* (2 species) and *Pereskia* (17 species) remain uncertain. Relationships among Opuntioideae, *Pereskia* and *Maihuenia* differ when different loci are analyzed, when different methods are used, and when different outgroup taxa are removed from analysis. Topologies differed more between loci using the same method than between method for the same locus. Bayesian analysis of *rbc*L strongly favors Opuntioideae sister to Blossfeldioideae-Cactoideae-Rhipsalidoideae and *Pereskia* united with *Maihuenia*. In contrast, analysis of *rpo*B strongly favors Opuntioideae as derived within a clade of some pereskias, whereas analysis of *mat*K cannot resolve with confidence the relationships

between Opuntioideae, *Maihuenia* and several *Pereskia* branches. In the combined analysis synapomorphies in *rpo*B outnumber changes on the short branches between the major basal clades and *rpo*B drives the phylogenetic signal.

From these analyses I conclude that 1) Opuntioideae is clearly monophyletic defined by numerous molecular synapomorphies evident in all gene partitions. 2) *Maihuenia* is a distinct lineage whose affinities can be defined by the partition or method of analysis chosen. 3) These data do not support a monophyletic *Pereskia*, but do identify three main branches within the genus congruent with those identified by Leuenberger (1986, p. 52). Placement of *Maihuenia* sister to the Blossfeldioideae-Rhipsalidoideae-Cactoideae clade in the parsimony analysis of combined data is supported by similar presence of stomata in pits (*Maihuenia*) and crypts (*Blossfeldia*) associated with the areole, and not consistent with a *Maihuenia*-Opuntioideae clade (Stevens, 2001 onwards [May, 2005]).

Different gene trees may appear incongruent due to a variety of causes other than differing underlying histories (Bull *et al.*, 1993; Huelsenbeck *et al.*, 1996). Rate heterogeneity among genes is well-documented (e.g., Delwiche & Palmer, 1996; Adachi et al., 2000). Furthermore, whole genome phylogenetic analysis has revealed that chloroplast protein-coding gene partitions for the same set of taxa typically result in discordant gene trees when constructed by the same method (Vogl et al., 2003) despite any evidence of paralogy, horizontal gene transfer, or recombination (Martin et al., 1998). Several studies have noted RNA polymerase genes as differing from most other chloroplast genes in evolutionary rate and amino acid composition (Martin et al., 1998; Lockhart et al., 1999; Vogl et al., 2003). For these markers in Cactaceae there is no evidence to suggest paralogy of gene copies or widespread problems due to lineage sorting, and base composition homogeneity was not refuted. Differences in covariotide

structure, ie. selection, could account for differences between these chloroplast genes. Rejection of congruence when true (Type I error) has been shown in ILD tests of data simulated using trees identical in topology but differing in branch lengths (Darlu & Lecointre, 2002; Dolphin *et al.* 2000; Barker & Lutzoni 2002).

Combining partitions with different rates of substitution can lead to less accurate estimation of phylogeny, especially when a homogeneous model of evolutionary process is applied erroneously (Bull *et al.*, 1993), but when phylogenetic signal is additive more robust estimates can result from combining heterogeneous data partitions (Sullivan, 1996). Analyses of the combined 3-gene data set highlights differences in the reconstruction of basal relationships using different optimality criteria (Figures 2.9, 2.10, 2.12). When different methods yield congruent tree topologies the results are generally accepted as robust even though different phylogenetic methods may agree on an incorrect phylogeny (Omilian & Taylor, 2001). In this case, neither maximum parsimony nor Bayesian analyses resolved the relationship of all *Pereskia* species with confidence. Only the Bayesian analysis joined the Andean *Pereskia* clade with the Opuntioideae with high confidence (99% probability). Therefore this result relies on confidence that the chosen model of evolution, GTR+%I+Γ) is adequate. If true, this result implies that stem succulence has arisen twice in the family!

Pereskia species appear to have diverged very rapidly early in the history of the family accumulating few synapomorphies for the genus. Several molecular characters in the *rpo*B partition are shared between Opuntioideae and the Andean *Pereskia* clade (including *P. aculeata* and *P. lychnidiflora*). Furthermore, a parametric bootstrap test confidently rejected the null hypothesis of *Pereskia* monophyly when simulated data sets were modeled using the same model of evolution (GTR+%I+Γ) used in phylogenetic analysis.

TEMPO OF MODE OF CACTACEAE EVOLUTION

Our results suggest that coding sequences from members of the Andean *Pereskia* clade and Opuntioideae are evolving faster than other pereskias, but at a similar rate to *Maihuenia, Blossfeldia* and the basal taxa of other clades in Cactoideae and Rhipsalidoideae. Previously, substitution rate variation within angiosperm families has not been extensively surveyed, although rates within grasses (Hudson et al., 1990; Gaut, Muse & Clegg, 1993), monocots (Gaut et al., 1992; Gaut et al., 1996) and across angiosperms (Bousquet et al., 1992; Gaut et al. 1993; Barraclough & Savolainen, 2001) have received attention. In a few studies adaptive selection has been detected in disease-resistance genes (R-genes: Bishop, Dean, and Mitchell-Olds, 2000; chitinases: Bergelson et al., 2001) and adaptive divergence has been suggested for the phytochrome genes in flowering plants (Mathews et al., 2003).

Lineage-specific evolutionary pressure on substitution rates are known to occur throughout the plastome (Gaut et al., 1993) leading to within-site rate variation, or heterotachy (Lopez, Casane & Phillipe, 2002). Changes in protein function that influence selection at specific nucleotide sites have been detected from studies of rate shifts[11]. However, the mechanisms of protein evolution that shape patterns of rate variation in the chloroplast are only beginning to be known (e.g., Adachi et al., 2000; Vogl et al., 2003). Two of the three genes used in this study are essential to photosynthesis, the primary means by which energy from the sun is converted to power all life on earth. The *rpo* genes encode core subunits of RNA polymerases that transcribe most photosynthesis-related genes (Suguira, 1992) but promoter-specificity factors for the enzyme appear to be encoded in the nuclear genome (Hanaoka et al, 2003) raising the question that *rpo*

[11] Shifts between any two rates, rather than a shift between variable and invariable as in a covarion model, are termed rate shift sites (Knudsen & Miyamoto, 2001).

genes might co-evolve with changes outside the chloroplast. Mechanisms for concentrating carbon around the RubisCo enzyme include CAM and C4 photosynthesis, pathways that are associated with Cactaceae and some Poaceae (grasses) respectively, and the adaptive mode of evolution. Both families are hypothesized to have radiated with the extension of warm arid landscapes after acquiring these physiological and associated morphological adaptations.

Rapid adaptive shifts and speciation

A strong correlation between the rate of gene sequence evolution, estimated from branch lengths, and number of species within a lineage has been shown for *rbc*L across flowering plants (Chase et al., 1993; Barraclough et al., 1996, but see also the re-analysis in Savolainen & Goudet, 1998). An increase in evolutionary rate appears among lineages of Cactaceae (probably) soon after the diversification of the family[12], and before the fully developed adaptive syndromes of stem succulence and CAM photosynthesis appear in the species of Opuntioideae, Cactoideae and Rhipsalidoideae. That the "fast" pereskias, *P. lychnidiflora*, *P. aculeata*, *P. horrida, P. weberiana* and *P. diaz-romeroana*, show significantly different rates than their congeners is a surprising result; on morphological grounds there is little reason to suspect that the genus is not monophyletic. Closely related to *Pereskia*, the two *Maihuenia* species have intermediate rates that bridge the 95% confidence intervals of both *Pereskia* groups. The two genera differ to a large degree in their habit and well-studied anatomical adaptations (Bailey, 1962, 1963; Eggli, 1984; Mauseth & Sajeva, 1992; Mauseth 1993a, 1993b,1999; Mauseth & Landrum, 1997; Nobel & Hartstock, 1988). The discovery of similar rates in

[12] Rates of outgroup taxa have not yet been examined; doing so requires a redefinition of the most recent common ancestor for the study.

Opuntioideae and the fast pereskias, and different rates between *Pereskia* species, focuses attention on common features and differences of these taxa.

Rapid invasion of a new adaptive zone is often associated with the appearance of a novel feature, or key innovation. The shift to stem succulence and photosynthesis is widely regarded as a key innovation for Cactaceae that allowed the radiation of the family into arid environments beyond the tropical deciduous forest habitats of most pereskias. In addition to several synapomorphies in *rpo*B, the fast pereskias share with Opuntioideae an orientation of guard cells perpendicular to their stomata (Eggli, 1984). In *Maihuenia* stems, axillary bud pits have persistent epidermis (lack of bark formation) with stomata[13], aerenchymous chlorenchyma, and as well as an effectively vascularized cortex that provide some supplement to leaf photosynthesis (Mauseth, 1999). *Maihuenia*'s water storage capacity and tissues are also generally more specialized than *Pereskia* (Mauseth, 1999). Delayed bark formation has recently been reported for some pereskias (Stevens, 2001 onwards [2005]), including most members of the Andean *Pereskia* clade that also show accelerated rates. Since bark inhibits stem-based photosynthesis, these anatomical features in the Andean *Pereskia* clade and *Maihuenia* could perhaps represent a preadaptation for stem photosynthesis and succulence in Opuntioideae, Blossfeldioideae, Cactoideae, and Rhipsalidoideae. The coincidence of changed rates and these changes in morphology from presumable plesiomorphic states in the "slow" pereskias make it tempting to assume that evolutionary and morphological rates are coupled. Whether or not molecular rates actually correlate with amounts of morphological change, as has been suggested for some groups (e.g., Omland, 1997), requires careful study of many morphological correlates and is beyond the immediate

[13] Although stomata are reported absent on stems by Eggli, 1984 and Leuenberger, 1997

scope of this study, particularly given the uncertainty of phylogenetic relationships precisely at the point of interest.

Rapid adaptive shifts have been associated with speciation events (Mayr, 1963; Eldredge & Gould, 1972; Stanley, 1979; Barraclough & Savolainen, 2001) and speciation has also been closely linked with rate of genetic change (Mayr, 1954; Harrison, 1991; Bousquet et al., 1992; Coyne, 1992; Savolainen & Goudet, 1998). Speciation events leading to the origin of new major groups is also one point in gene evolution where functional change is most likely (Knudsen & Miyamoto, 2001; Moreira, Le Guyader, & Phillipe, 2001; Mathews et al., 2003). Substitution rate increases that may herald functional shifts in one or more of the protein-coding genes sequenced here do precede the diversification of the two largest genera of Cactaceae, *Mammillaria* and *Opuntia*. *Mammillaria* represents a core lineage whose sister group comprises far fewer species. The possible sister taxon of *Opuntia* is probably either a small group of pereskias or that together represent only 5% of the species number present in Opuntioideae. Of much interest for future study is the more recent case of rate increase associated with the largest genus of Cactaceae, *Mammillaria,* and allies, that provide a rich system for population level studies of diversification.

Theory predicts that an evolutionary rate increase accompanies the seemingly abrupt appearance of novel adaptations marking the origin of new taxa at relatively higher rank. The change in relative rates as reflected in branch lengths, together with the phylogenetic results presented here, show that an increase in evolutionary rate has occurred coincident with the acquisition of incipient adaptive traits for which the Cactaceae is renowned. If any of the genes surveyed here had played a role in the adaptation of Cactaceae to open arid environments, then we would also expect to find evidence of altered selective constraints. Extension of this project to confirm the

presence of selection at the molecular level by partitioning the total substitution rate by locus and into synonymous and non-synonymous components is planned. Lineage-specific substitution rates observed in Cactaceae may bias phylogenetic analysis under homogenous models of DNA sequence evolution, but they also focus our attention more acutely on possible biological causes for morphological disjunction. These results from Cactaceae provide another example and well sampled data set that contributes evidence in support of a synthetic theory of adaptive evolution.

Chapter 3: What is a *Mammillaria*?

INTRODUCTION

Mexico, because of its geographic position, climate and complex orography has a myriad of microclimates that have given rise to one of the richest floras in the world. Seasonally dry forests and deserts occupy most of the territory of Mexico and these areas are home to a great number of species of the families Poaceae, Asteraceae, and Cactaceae for which Mexico is an important center of diversity. The approximately 700 taxa of the Cactaceae in Mexico represent about 3% of the vascular flora of that country. More than half the species of Mexican Cactaceae belong to subfamily Cactoideae which is essentially endemic to Mexico. Among the most widespread and speciose genera of Cactoideae is *Mammillaria* Haw. with species are equally at home in the deserts of northern Mexico or on rocky outcrops in the tropical and subtropical pine or pine-oak forests covering most of the mountainous regions of the country. The many species of *Mammillaria* dotted across the Mexican landscape provide a bewildering array of morphological variation in their pincushion-like habit and flowers. This diversity of morphological traits translates into a complex taxonomy that has confounded cactus specialists for centuries providing widely divergent estimates for its component species ranging from 145 to more than 300. Because of the vagaries of history, the original description of the genus *Mammillaria* is based on one of only two or three species found south of Mexico in small populations hugging the Caribbean coast and reaching eastern Venezuela.

The Cactoideae as presently understood (Crozier, 2004) constitute a monophyletic lineage that represents an outstanding radiation in the deserts of North America from an apparent long-distance dispersal event from South America (Chapter 2). Most genera

and species are found in the tropical and subtropical eastern half of the Chihuahuan Desert (Henrickson, unpublished). As shown in Figure 1, the classification and taxonomic history of the Cactoideae have gravitated around the taxonomic limits of *Mammillaria* and *Echinocactus* Link & Otto and the various interpretations of the circumscriptions of these taxa. Schumann (1899) considered *Mammillaria* and *Echinocactus* two large genera that contained several important lineages he recognized at the subgenus level. Britton and Rose (1919-1923) elevated some of Schumann's subgenera to generic status and described several additional new genera. Their confidence in their bold new taxonomy was supported by access to a much improved, larger set of specimens and field experience. Subsequent to the publication of their studies, an incipient peace in Mexico after the revolutionary years fostered exploration of the northern deserts producing a bounty of new collections, the basis of a plethora of new genera. After Britton & Rose, most of the taxonomic pronouncements in the family and subfamily Cactoideae, along with a concomitant increase in the number of genera and taxonomic complexity, are dominated by Backeberg (1958-62) and Buxbaum (1951a). This period of species and generic expansion was followed by consolidation in the studies of Barthlott & Hunt (1993) who strongly reduced the number of genera of Cactoideae and circumscribed the subfamily (as tribe Cacteae) to include only those taxa of mostly North American distribution having floral parts without areoles and associated spines. Their studies brought stability to the classification of the Cactoideae but doubts remained as to the taxonomic limits of the genus *Mammillaria*.

Mammillaria is the largest genus of Cactaceae. Historically it has been viewed as the main lineage of a complex of genera sharing tuberculate podaria that includes *Coryphantha*, *Escobaria*, *Neolloydia* and *Ortegocactus,* among others. These genera and

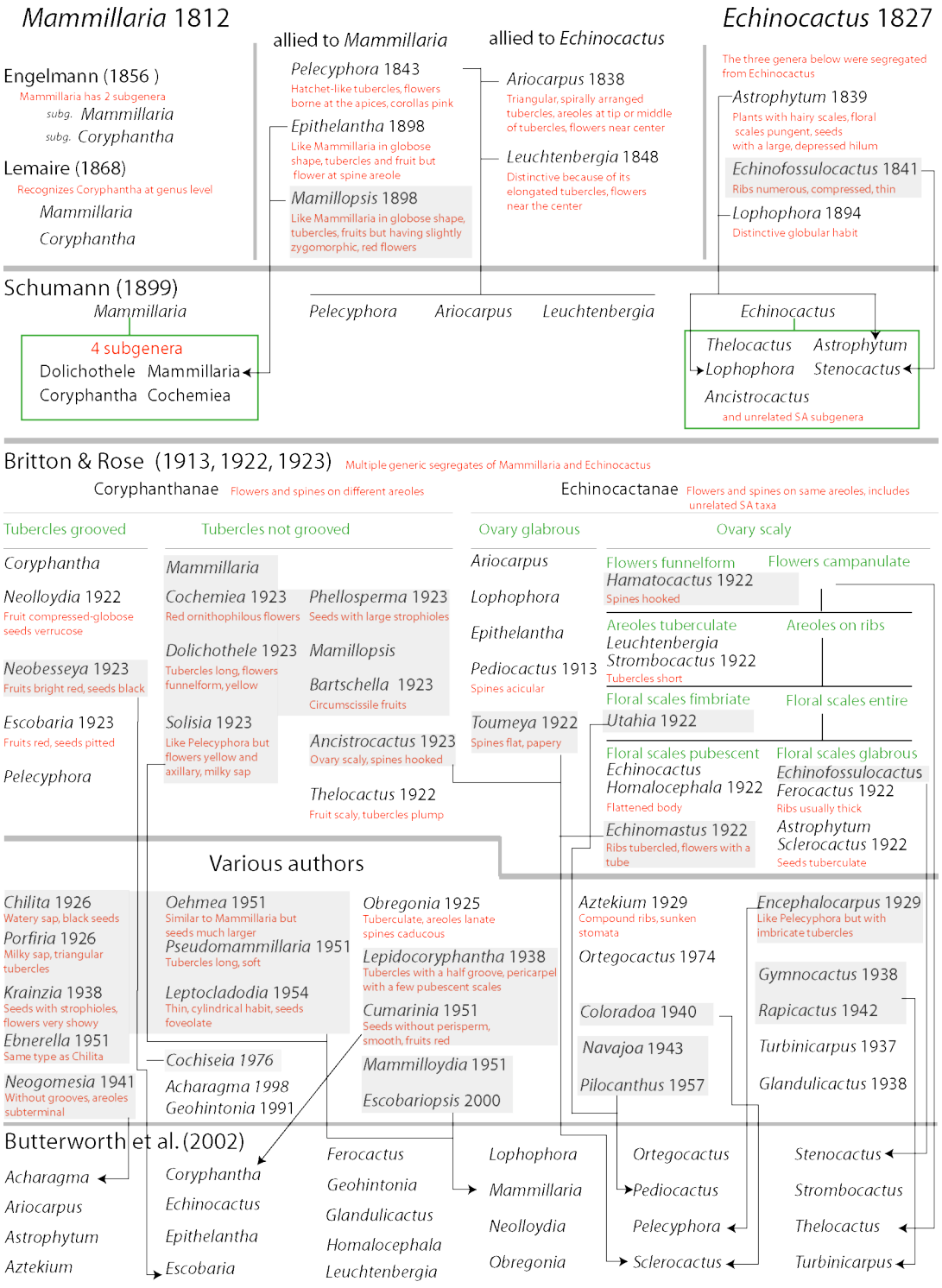

Figure 3.1. Diagrammatic view of the taxonomic history of the Cactoideae.

some other segregates of *Mammillaria* have a complex taxonomic history whose common denominator has been to be a synonym of one another, or of the perceived core genus *Mammillaria*. *Mammillaria* was the first genus in the complex to be named. Its taxonomic history is closely intertwined with that of the family since *Mammillaria* is the conserved type of Cactaceae (Greuter et al., 2000). Linnaeus (1753) was the first to recognize a species of *Mammillaria* as *Cactus mammillaris*. Subsequently, Haworth (1812) used this taxon as the basis of his genus *Mammillaria* and the type species designated as *M. simplex* (now *M. mammillaris)*. Lemaire (1839) viewed *Mammillaria* as a genus of 4 lineages. His section *Aulacothelae* contained the species we recognize today as coryphanthas. Lemaire's views on *Mammillaria* were probably influenced by the opinions of Pfeiffer (1837) who introduced informal subdivisions within *Mammillaria* but without mention as to taxonomic rank. Engelmann (1856) proposed *Mammillaria* subgenus *Coryphantha* based on the morphology of the areoles, a decision that apparently Lemaire (1868) strongly accepted because he raised subgenus *Coryphantha* to genus level. This action set the stage for the hypothesis that *Coryphantha* represents the sister taxon of *Mammillaria*.

The sections of *Mammillaria* recognized by Lemaire were mostly based on differences in habit and podarium/tubercle morphology, as opposed to the classification by Pfeiffer that was based mostly on spine characteristics. In the interim, Salm Dyck (1850) created a classification for *Mammillaria* that included 11 sections and 6 subsections based on his extensive (for the time) living collection. Schumann (1899) summarized the views of his predecessors accepting some of their sectional groupings; later these were lectotypified by Hunt (1971, 1977a,b,c). Schumann viewed *Mammillaria* as a genus with 4 subgenera including subgenus *Mammillaria* (as *Eumammillaria*) having 2 sections and 15 series, subgenus *Coryphantha,* and the new

subgeneric segregates *Cochemiea* and *Dolichothele*. The two sections of *Mammillaria* were based on the nature of the sap: watery in *M*. sect. *Hydrochylus*; milky in *M*. sect. *Galactochylus*. This character was impossible to observe in herbarium specimens leading to uncertainty in the classification and placement of new species. This situation was exacerbated as many new species began to be named from novelties resulting from ever more ambitious fieldtrips into the Mexican deserts. *Cochemiea* was viewed as distinctive because of its red, ornithophilous flowers whereas *Dolichothele* species have large tubercles and showy funnel-shaped yellow flowers with a solid tube.

Britton and Rose (1919-1923) in their monograph of the Cactaceae overhauled the taxonomy of the family producing numerous new genera in Cactoideae including several carved out of traditional *Mammillaria* or *Coryphantha* (Figure 3.1). *Bartschella* was thought to be different from *Mammillaria* or *Coryphantha* because of its circumscissile fruits. *Escobaria* was presumed close to *Coryphantha* but with distinctive fruits and seeds. *Neobesseya* was believed closest to *Coryphantha*, but like *Escobaria* its fruits and seeds are different. *Neolloydia*, like *Coryphantha* has deeply grooved tubercles but nearly glabrous ovaries. *Phellosperma* was considered distinct because of its corky, strophiolate seeds. The genus *Solisia* was segregated from *Pelecyphora* because of its milky sap, yellow instead of pink corollas, and lateral rather than central flowering. For several workers of the Cactoideae including Zimmerman (1985) and Lüthy (1995) the decisions of Britton & Rose concerning the relationships of *Mammillaria* and relatives produced a complex taxonomy that obscured true phylogenetic relationships, in essence, a lack of progress.

Buxbaum (1950, 1951a, b) considered *Mammillaria* a lineage originating from *Coryphantha*. He believed that the genera previous workers recognized as sister to, or presumed derived from *Mammillaria* had attained a similar stage of ontogenetic

development along parallel evolutionary lines. He recognized three lineages in tribe Cacteae all evolving towards a morphology that showed simplicity or reduction in habit and flower structure. Genera were organized along 5 ontogenetic stages and those at higher stages were presumed to have evolved directly from those genera at lower levels, an idea not easily reconciled with modern views of evolution. In 1956, he came to view *Mammillaria* as a more inclusive entity nevertheless maintained his generic segregates *Mammilloydia*, *Oehmea*, and *Cumarinia* for *Corypantha macromeris* (Figure 3.1).

In a series of papers Hunt (1971, 1977 a,b,c,d, 1981, 1999) clarified the taxonomy and classification of *Mammillaria*. Arguably, his most important contribution resides in having typified the infrageneric names (series) of Schumann (1897-99) and merging with this classification some of the phylogenetic ideas of Buxbaum. His classification emphasizes practicality and the use of the geographical distribution of plants, along with morphology, to circumscribe infrageneric groups. His latest classification (1981) recognizes 6 subgenera, with subgenus *Mammillaria* containing 3 sections and 14 series (Figure 3.2). Along with these studies he also evaluated all the names in *Mammillaria* reducing the number of species to approximately 168.

Hunt's studies were evaluated by Lüthy (1995, 2001) who produced a similarly clear classification of the genus, resurrecting the sectional names of Lemaire (1839), and making some nomenclatural adjustments to the taxonomy. Lüthy (1995) recognized *Mammilloydia* at generic rank. The largest area of disagreement between these two workers is in the composition of *M.* sect. *Hydrochylus* (sect. *Cylindricothelae*, Lüthy, 1995) that according to both workers probably contains the basal elements of the genus. Lüthy did not recognize a *M.* sect. *Ancistracanthae*. Rather he believed most of its species to be part of his concepts of *M.* subg. *Cochemiea* and *Phellosperma*. Both

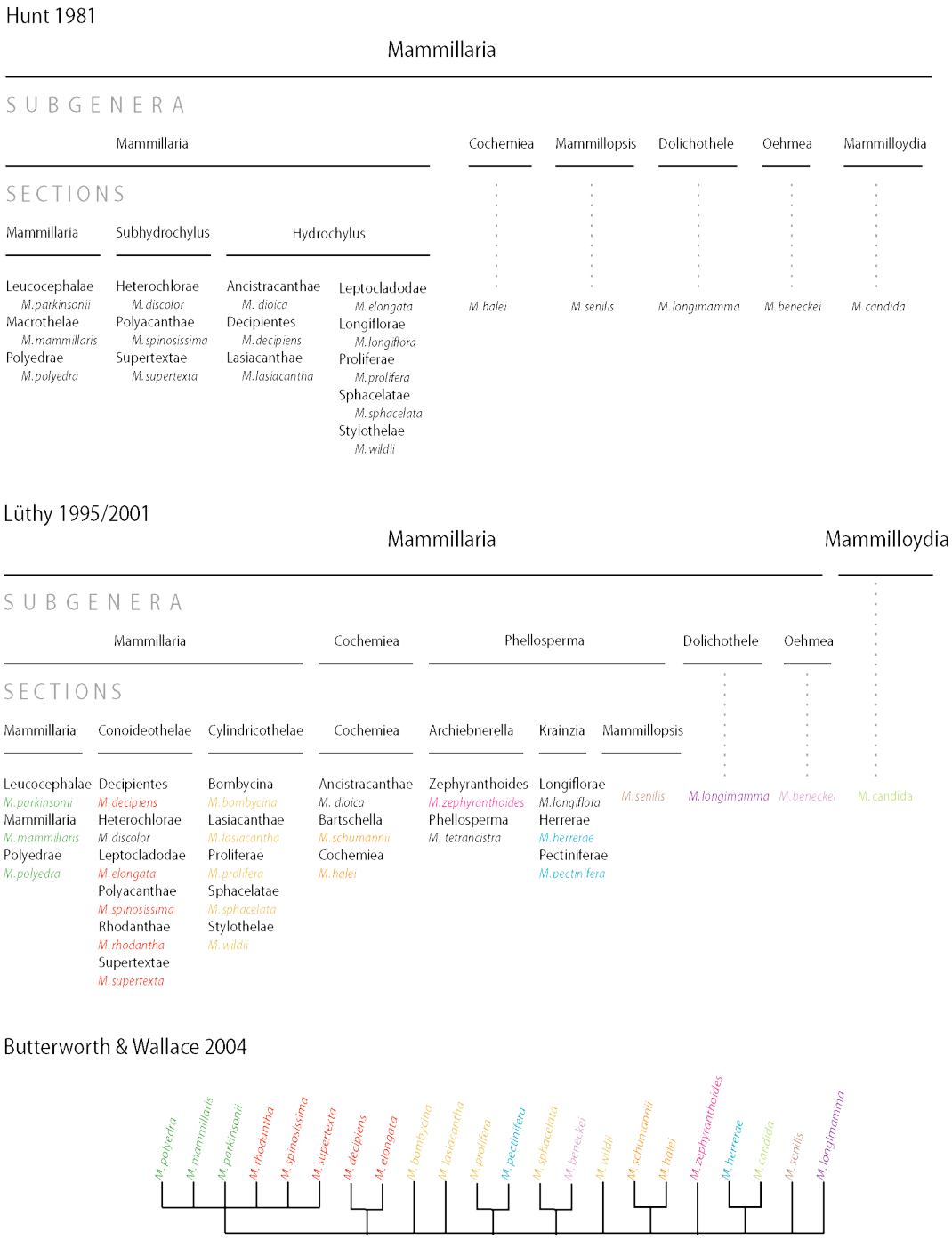

Figure 3.2. Comparison of classification schemes by Hunt (1981) and Lüthy (1995, 2001) for *Mammillaria* vs molecular phylogeny of Butterworth & Wallace 2004 based on noncoding regions of the cpDNA.

classifications are practical, and easy to use, and provide a hypothesis of relationship that constitute a phylogeny for the genus *Mammillaria*. Both authors believe *Mammillaria* to be a monophyletic entity sister to *Coryphantha* and related genera.

Zimmerman (1985), in his detailed account of the morphology and taxonomic history of *Coryphantha*, believed *Mammillaria* to be inextricably connected to *Coryphantha*. The technical differences or key characters separating *Coryphantha* from *Mammillaria* and its related genera are all tenuous, and Zimmermann (1985) hinted at the possibility of an all-encompassing genus *Mammillaria*.

Molecular studies were initiated to test the classifications of Hunt (1981) and Lüthy (1995, 2001). During the course of this study, Butterworth & Wallace (2004) published a molecular phylogeny of the genus based on the *rpl*16 intron and the *psb*A-*trn*H intergenic spacer regions of the chloroplast DNA. Their study included 125 taxa (113 mammillarias) and was aimed at testing the classification of *Mammillaria* based on Hunt and Lüthy. In addition, another important goal of their study was to identify the phylogenetic relationships of several genera that historically have been viewed as closely related to *Mammillaria* (namely *Coryphantha*, *Escobaria*, *Neolloydia* and *Ortegocactus*). For several reasons, ranging from the choice of outgroups to the quality and amount of data needed to resolve strongly supported clades, their study failed to answer most of the questions they posed. Therefore, the main questions pursued at the inception of our study are still pertinent:

1) What is a *Mammillaria*? 2) What are the sister genera of *Mammillaria*? 3) Which groups of *Mammillaria* are the basalmost lineages of the genus? 4) Can our molecular phylogeny refine the classifications of Hunt (1981) or Lüthy (1995, 2001)?

To answer these questions we undertook a study that includes most of the nomenclaturally significant species of *Mammillaria*, most genera of Cactoideae plus

representatives of the other 5 subfamilies of Cactaceae. Our study is based solely on sequences of the chloroplast genome including the 3 protein-coding genes, 3 intergenic spacer regions, and 4 Group II introns, sampled from each of the 157 genera included in the study.

MATERIALS AND METHODS

Taxon and Character Sampling

In addition to the 113 taxa sampled in the coding region study (Chapter 2) an additional 44 species of *Mammillaria* and allies were sampled (Table 3.1) in an attempt to sample all the sections and series of Hunt's (1981) and Lüthy's (1995) *Mammillaria* classifications. Because delimiting genera in Cactaceae is difficult and subject to broad disagreement, type species and genera were sampled whenever possible so that these results might inform taxonomic revisions. In previous experiments it was determined that additional taxon samples outside the area of immediate interest (*Mammillaria* and allies) improved the robustness of our phylogenetic results, so all cactus samples were included in this study. Voucher specimens are deposited in the countries of origin where appropriate, and/or at the Plant Resources Center at the University of Texas (TEX).

Table 3.1. List of taxa used in the molecular study. Vouchers deposited at TEX.

TAXON	VOUCHER #	ORIGIN
Acharagma aguirreana (Glass & Foster) Glass	*Crozier DISS1*	Mexico
Acharagma roseanus (Boedeker) E. F. Anderson	*Crozier DISS2*	Mexico

Alluaudia dumosa Drake	*Crozier DISS16*	Madagascar
Anacampseros kurtzii Bacigalupo	*Crozier DISS17*	Argentina
Aporocactus flagelliformis (L.) Lemaire	*Crozier DISS3*	Mexico
Arequipa rettigii (Quehl) Oehme	*Crozier DISS102*	Peru
Ariocarpus retusus Scheidweiler	*Crozier DISS4*	Mexico
Arrojadoa penicillata (Guerke) Britton & Rose	*Crozier DISS160*	Brazil
Astrophytum myriostigma (Karwinsky ex Zucc.) Lem.	*Crozier DISS6*	Mexico
Austrocactus patagonicus (Weber ex Speg.) Hosseus	*Crozier DISS18*	Argentina
Austrocylindropuntia subulata (Berger) Backeb.	*Crozier DISS19*	Bolivia
Aztekium hintonii Glass & Fitz Maurice	*Crozier DISS7*	Mexico
Aztekium ritteri (Boedeker) Boedeker	*Crozier DISS8*	Mexico
Basella alba L.	*Crozier DISS20*	USA
Blossfeldia liliputana Werdermann	*Crozier DISS21*	Argentina
Browningia candelaris (Meyen) Britton & Rose	*Crozier DISS9*	Peru
Calymanthium substerile Ritter	*Crozier DISS11*	Peru
Carnegiea gigantea (Engelmann) Britton & Rose	*Crozier DISS12*	USA
Cephalocereus senilis (Haworth) Pfeiffer	*Crozier DISS13*	Mexico
Cereus jamacaru DC	*Crozier DISS22*	Brazil
Cintia knizeii Riha	*Crozier DISS14*	Bolivia
Cleistocactus baumannii (Lemaire) Lemaire	*Crozier DISS15*	Brazil
Consolea rubescens (Salm-Dyck) Lemaire	*Crozier DISS23*	Puerto Rico
Copiapoa cinerea (Philippi) Britton & Rose	*Crozier DISS24*	Chile
Copiapoa marginata (Salm-Dyck) Britton & Rose	*Crozier DISS25*	Chile
Corryocactus brevistylus (Schumnann) Britton & Rose	*Crozier DISS26*	Peru
Coryphantha macromeris (Engelmann) Lemaire	*Crozier DISS27*	USA
Coryphantha missouriensis (Sweet) Britton & Rose	*Crozier DISS42*	USA
Coryphantha odorata Boedeker	*Crozier DISS28*	USA
Coryphantha robbinsorum (Earle) A. Zimmerman	*Crozier DISS43*	USA
Coryphantha sulcata (Engelmann) Britton & Rose	*Crozier DISS29*	USA
Cylindropuntia imbricata (Haw.) F. Knuth	*Crozier DISS30*	USA
Denmoza rhodocantha (Salm-Dyck) Britton & Rose	*Crozier DISS31*	Argentina
Discocactus placentiformis (Lehmann) Schumann	*Crozier DISS32*	Brazil
Disocactus biformis (Lindley) Lindley	*Crozier DISS33*	Guatemala
Echinocactus platyacanthus Lemaire	*Crozier DISS34*	Mexico
Echinocactus texensis Hopffer	*Crozier DISS35*	USA
Echinocereus viridiflorus Engelmann	*Crozier DISS36*	USA
Echinopsis eyriesii (Turpin) Pfeiffer & Otto	*Crozier DISS37*	Argentina
Echinopsis macrogona (Salm-Dyck) Friedrich & Rowley	*Crozier DISS161*	Bolivia
Epiphyllum phyllanthus (L.) Haworth	*Crozier DISS38*	Brazil

Epithelantha micromeris (Engelmann) Weber	*Crozier DISS39*	USA
Eriosyce islayensis (Foerster) Kattermann	*Crozier DISS40*	Peru
Eriosyce subgibbosa (Haworth) Kattermann	*Crozier DISS41*	Chile
Escobaria tuberculosa (Engelmann) Britton & Rose	*Crozier DISS44*	USA
Escontria chiotilla (Weber ex Schumann) Rose	*Crozier DISS45*	Mexico
Eulychnia breviflora Philippi	*Crozier DISS46*	Chile
Ferocactus wislizeni (Engelmann) Britton & Rose	*Crozier DISS48*	USA
Frailea cataphracta (Dams) Britton & Rose	*Crozier DISS49*	Brazil
Geohintonia mexicana Glass & Ftiz Maurice	*Crozier DISS50*	Mexico
Glandulicactus uncinatus (Galeotti) Backeberg	*Crozier DISS51*	Mexico
Gymnocalycium denudatum (Link & Otto) Pfeiffer ex Miller	*Crozier DISS52*	Brazil
Haageocereus pseudomelanostele (Werdermann & Backberg) Backberg	*Crozier DISS53*	Peru
Halophytum ameghinoi Speg.	*Crozier DISS54*	Argentina
Hatiora salicornioides (Haworth) Britton & Rose ex Bailey	*Crozier DISS55*	Brazil
Hylocereus triangularis (L.) Britton & Rose	*Crozier DISS56*	Jamaica
Leptocereus quadricostatus (Bello) Britton & Rose	*Crozier DISS57*	Puerto Rico
Leuchtenbergia principis Hooker	*Crozier DISS58*	Mexico
Lophophora williamsii (Lemaire ex Salm-Dyck) J. Coulter	*Crozier DISS59*	USA
Maihuenia patagonica (Philippi) Spegazzini	*Crozier DISS60*	Argentina
Maihuenia poeppigii (Pfeiffer) Schumann	*Crozier DISS61*	Argentina
Maihueniopsis glomerata (Haw.) Kiesling	*Crozier DISS62*	Argentina
Mammillaria beneckei Ehrenberg	*Crozier DISS63*	Mexico
Mammillaria crinita (DC.) ssp. Wildii (A. Dietrich) D. R. Hunt	*Crozier DISS65*	Mexico
Mammillaria crucigera Martius	*Crozier DISS66*	Mexico
Mammillaria decipiens Scheidw. ssp. Camptotricha (Dams) D. R. Hunt	*Crozier DISS67*	Mexico
Mammillaria dioica K. Brandegee	*Crozier DISS68*	Mexico
Mammillaria discolor Haw.	*Crozier DISS69*	Mexico
Mammillaria elongata DC	*Crozier DISS70*	Mexico
Mammillaria geminispina Haworth	*Crozier DISS71*	Mexico
Mammillaria halei Brandegee	*Crozier DISS72*	Mexico
Mammillaria heyderi Muehlenpfordt	*Crozier DISS73*	USA
Mammillaria lasiacantha Engelmann	*Crozier DISS74*	USA
Mammillaria longiflora (Britton & Rose) Berger	*Crozier DISS75*	Mexico
Mammillaria longimamma DC	*Crozier DISS76*	Mexico
Mammillaria mammillaris (L.) Karsten	*Crozier DISS77*	Venezuela

Mammillaria napina Purpus	*Crozier DISS78*	Mexico
Mammillaria parkinsonii Ehrenberg	*Crozier DISS79*	Mexico
Mammillaria pectinifera Weber	*Crozier DISS80*	Mexico
Mammillaria polyedra Martius	*Crozier DISS81*	Mexico
Mammillaria polythele Martius	*Crozier DISS82*	Mexico
Mammillaria prolifera (Miller) Haworth	*Crozier DISS83*	USA
Mammillaria rhodantha Link & Otto	*Crozier DISS84*	Mexico
Mammillaria schumannii Hildmann	*Crozier DISS85*	Mexico
Mammillaria senilis Loddiges ex Salm-Dyck	*Crozier DISS86*	Mexico
Mammillaria sphacelata Martius	*Crozier DISS87*	Mexico
Mammillaria spinosissima Lemaire	*Crozier DISS88*	Mexico
Mammillaria supertexta Martius ex Pfeiffer	*Crozier DISS89*	Mexico
Mammillaria theresae Cutak	*Crozier DISS90*	Mexico
Mammilloydia candida (Scheidweiler) Buxbaum	*Crozier DISS91*	Mexico
Matucana haynei (Otto ex Salm-Dyck) Britton & Rose	*Crozier DISS92*	Peru
Melocactus caroli-linnaei Taylor	*Crozier DISS93*	Jamaica
Mila caespitosa Britton & Rose	*Crozier DISS94*	Peru
Myrtillocactus geometrizans (Martius) Console	*Crozier DISS95*	Mexico
Neobuxbaumia mezcalensis (Bravo) Backeberg	*Crozier DISS96*	Mexico
Neolloydia conoidea (DC) Britton & Rose	*Crozier DISS97*	USA
Neoraimondia arequipensis (Meyen) Backeb.	*Crozier DISS98*	Peru
Obregonia denegrii Fric	*Crozier DISS99*	Mexico
Opuntia macrocentra Engelmann	*Crozier DISS100*	USA
Oreocereus celsianus (Salm-Dyck) Riccobono	*Crozier DISS101*	Peru
Oroya peruviana (Schumann) Britton & Rose	*Crozier DISS103*	Peru
Ortegocactus macdougallii Alexander	*Crozier DISS104*	Mexico
Pachycereus pringlei (Watson) Britton & Rose	*Crozier DISS105*	Mexico
Pachycereus schottii (Engelmann) D. R. Hunt	*Crozier DISS106*	Mexico
Parodia microsperma (Weber) Speg.	*Crozier DISS107*	Argentina
Parodia ottonis (Lehmann) Backeb.	*Crozier DISS108*	Argentina
Pediocactus simpsonii (Engelmann) Britton & Rose	*Crozier DISS109*	USA
Pelecyphora aselliformis Ehrenberg	*Crozier DISS110*	Mexico
Pelecyphora strobiliformis (Werdermann) Fric & Sch	*Crozier DISS111*	Mexico
Peniocereus greggii (Engelmann) Britton & Rose	*Crozier DISS112*	Mexico
Peniocereus striatus (Brandegee) Buxbaum	*Crozier DISS113*	Mexico
Pereskia aculeata Miller	*Crozier DISS114*	Mexico
Pereskia bahiensis Guerke	*Crozier DISS115*	Brazil
Pereskia bleo (Kunth) DC	*Crozier DISS116*	Panama
Pereskia diaz-romeroana Cárdenas	*Crozier DISS116*	Bolivia
Pereskia grandifolia Haworth	*Crozier DISS117*	Brazil
Pereskia guamacho Weber	*Crozier DISS118*	Venezuela

Pereskia humboldtii Britton & Rose	*Crozier DISS119*	Peru
Pereskia lychnidiflora DC	*Crozier DISS120*	Mexico
Pereskia nemorosa Rojas	*Crozier DISS121*	Argentina
Pereskia portulacifolia (L.) Haw.	*Crozier DISS122*	Hispaniola
Pereskia quisqueyana Liogier	*Crozier DISS123*	Hispaniola
Pereskia sacharosa Griseb.	*Crozier DISS124*	Argentina
Pereskia stenantha Ritter	*Crozier DISS125*	Brazil
Pereskia weberiana Schumann	*Crozier DISS126*	Bolivia
Pereskia zinniiflora DC	*Crozier DISS127*	Hispaniola
Pereskiopsis porteri (Brandegee) Buxbaum	*Crozier DISS128*	Mexico
Pfeiffera ianthothele (Monville) Weber	*Crozier DISS129*	Bolivia
Phemeranthus calycinus (Engelm.) Kiger	*Crozier DISS130*	USA
Pilosocereus alensis (Weber ex Grosselin) Byles & Rowley	*Crozier DISS131*	Mexico
Portulaca sp	*Crozier DISS133*	USA
Portulacaria afra Jacq.	*Crozier DISS134*	South Africa
Pterocactus tuberosus (Pfeiffer) Britton & Rose	*Crozier DISS135*	Argentina
Quiabentia verticillata (Vaupel) Vaupel	*Crozier DISS136*	Argentina
Rebutia minuscula Schumann	*Crozier DISS137*	Argentina
Rhipsalis baccifera (J. S. Mueller) Stearn	*Crozier DISS138*	Mexico
Sclerocactus papyracanthus (Engelm.) N. P. Taylor	*Crozier DISS139*	USA
Sclerocactus polyancistrus (Engelm. & Bigelow) Britton & Rose	*Crozier DISS140*	USA
Sclerocactus scheeri (Salm-Dyck) N. P. Taylor	*Crozier DISS141*	USA
Selenicereus sp.	*Crozier DISS142*	Mexico
Stenocactus coptonogonus (Lemaire) Berger ex Hill	*Crozier DISS143*	Mexico
Stenocereus alamosensis (J. Coulter) Gibson & Horak	*Crozier DISS144*	Mexico
Stenocereus stellatus (Pfeiffer) Riccobono	*Crozier DISS145*	Mexico
Stenocereus thurberi (Engelmann) Buxbaum	*Crozier DISS146*	Mexico
Stephanocereus leucostele (Guerke) Berger	*Crozier DISS147*	Brazil
Stetsonia coryne (Foerster) Britton & Rose	*Crozier DISS148*	Argentina
Strombocactus disciformis (DC.) Britton & Rose	*Crozier DISS149*	Mexico
Talinella pachypoda U. Eggli	*Crozier DISS150*	Madagascar
Talinum paniculatum (Jacq.) Gaertn.	*Crozier DISS151*	Peru
Tephrocactus inermis (Speg.) Backeb.	*Crozier DISS152*	Argentina
Thelocactus hexaedrophorus (Lemaire) Britton & Rose	*Crozier DISS153*	Mexico
Thelocactus setispinus (Engelmann) Anderson	*Crozier DISS154*	Mexico
Turbinicarpus saueri (Boedeker) John & Riha	*Crozier DISS155*	Mexico
Turbinicarpus schmiedickeanus (Boedeker) Buxbaum	*Crozier DISS156*	Mexico

& Backeberg		
Uebelmannia gummifera (Backeberg & Voll) Buining	*Crozier DISS157*	Brazil
Weberbauerocereus weberbaueri (Schumann ex Vaupel) Backeberg	*Crozier DISS158*	Peru
Yavia cryptocarpa Kiesling & Piltz	*Crozier DISS159*	Argentina

To infer a plastid phylogeny for these 157 species, the *rpl*16, *trn*K, and *rpo*C1 introns, and *trn*K-*psb*A, *rpl*20-*rps*12, *trn*L-F, and *trn*T-*trn*L intergenic spacer regions were sequenced. All these non-coding markers have been used widely in plant systematic studies previously, proving useful characters for interspecific studies. In addition three genes, *mat*K, *rbc*L, and *rpo*B, used in a previous study of Cactaceae by the author were sequenced for the 44 additional species in this study (Figure 3.3).

My sampling strategy reflects the premise that non-coding regions of the chloroplast DNA would yield enough informative characters to resolve most of the clades of the Cactaceae. In practical terms, I wanted our data to be useful to other workers in the Cactaceae who have extensively used non-coding sequences and/or the presence or absence of chloroplast introns to elucidate phylogenetic relationships in the family (Wallace, 1995a; Butterworth et al., 2004, and references therein). So I also sequenced popular non-coding markers and combined this approach with sequencing multiple coding regions of the chloroplast that were deemed to evolve more slowly. Of the total 13,799 aligned base pairs 1,061 characters were deemed ambiguously aligned and were excluded from analysis, resulting in a matrix of 12,738 bp for each of the 157 genera included in the study (Table 3.2). More than half (~52%) of the total data set represents non-coding data.

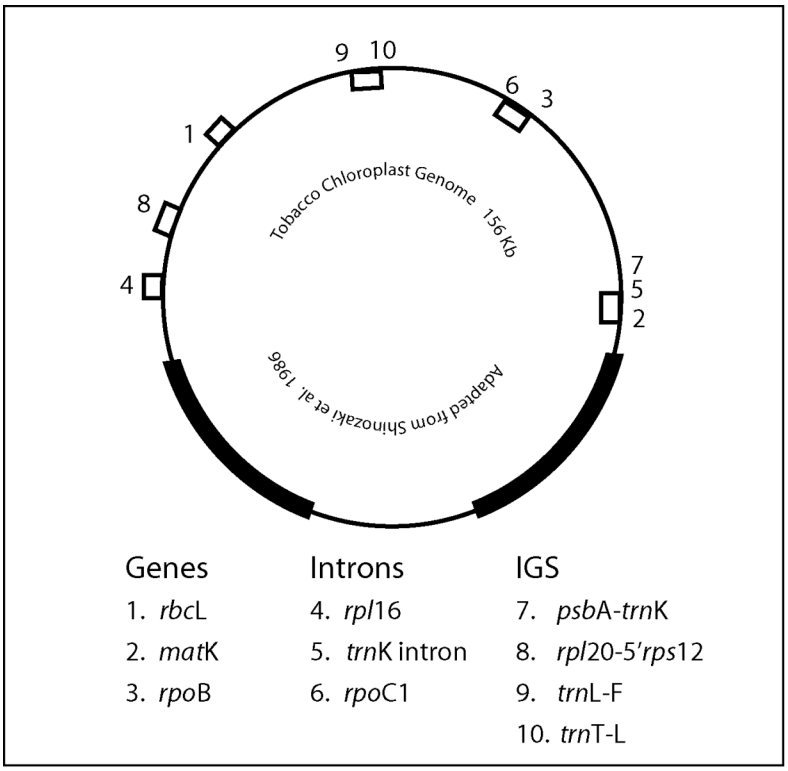

Figure 3.3. Map of the chloroplast DNA of tobacco with regions sequenced for this study.

DNA purification and sequencing

Total DNA was extracted from stem or leaf tissue of living or herbarium specimens using the organelle pellet method of Scott & Playford (1996). *Mammillaria* and allied taxa presented no exceptional barriers to DNA extraction, although an additional phenol-cleaning step was used for extremely mucilaginous samples. As little

Table 3.2. Summary of base composition and nucleotide substitution process statistics.

Partition name	# sites	# sites analysed	variable sites	MPinfo sites	Chi^2 Test of Base Frequency Homogeneity (df=363)	Chi^2 Test of Molecular Clock	% *Invariant*	α	ModelTest hLRT	ModelTest AIC
*mat*K	1575	1527	568	278	X^2 = 21.020230 P = 1.00000000	P<<0.001	0	0.6485	TVM+I+Γ	TVM+I+Γ
*trn*K exon 2	35	35	9	2	X^2 = 14.191512 P = 1.00000000		0	0.6198	JC	TVM
*rbc*L	1476	1320	321	177	X^2 = 11.143335 P = 1.00000000	P<<0.001	0.5024	0.9168	TIM+I+Γ	GTR+I+Γ
*rpo*B	3336	3156	1043	547	X^2 = 23.712553 P = 1.00000000	P<<0.001	0.3625	0.8229	GTR+I+Γ	GTR+I+Γ
GENES	***6422***	***6008***	***1931***	***1002***			0.3867	0.9078	GTR+I+Γ	GTR+I+Γ
*trn*K intron	1117	1105	358	171	X^2 = 17.357201 P = 1.00000000		0.2816	1.0558	K81uf+Γ	K81uf+I+Γ
*rpl*16 intron	1925	1674	620	304	X^2 = 399.518644 P = 0.09062398		0.1148	0.8131	K81uf+Γ	GTR+I+Γ
*rpo*C1 intron	946	946	148	42	X^2 =10.261600 P = 1.00000000		0	0.3575	K81uf+Γ	TVM+Γ
INTRONS	***3988***	***3725***	***1126***	***517***						
*trn*K-*psb*A IGS	344	324	148	92	X^2 = 44.569283 P = 1.00000000		0	0.7768	F81+Γ	GTR+Γ
*rpl*20-*rps*12 IGS	1033	997	279	137	X^2 = 28.219956 P = 1.00000000		0.2157	0.8681	GTR+I+Γ	GTR+I+Γ
*trn*L-*trn*F IGS	518	504	194	106	X^2 = 47.202944 P = 1.00000000		0	1.5545	TVM+Γ	TVM+Γ
*trn*T-*trn*L IGS	1494	1193	379	168	X^2 = 378.840895 P = 0.27284636		0	0.9294	TVM+I+Γ	K81uf+Γ
IGS	***3389***	***3018***	***1000***	***503***						
NONCODING	***7377***	***6730***	***2126***	***1020***						
Total	**13,799**	**12,738**	**3,128**	**2,022**			0.3240	0.8700	**TVM+I+Γ**	**TVM+I+Γ**

as 0.19 g tissue provided enough DNA for amplification of all markers as well as experimentation, an important consideration when receiving small samples of rare or governmentally regulated species. DNA was amplified by PCR, purified, and sequenced as described in Panero & Crozier (2003); primers are listed in Table 3.3. All sequences were produced *de novo* for this study at the DNA core facility or the Hillis-Bull Lab at the University of Texas at Austin using standard methods as referred to in Panero & Crozier (2003).

Table 3.3. List of primers used to amplify DNA.

Markers	5’ -3’ primer sequence	Reference
***rpo*B-*rpo*C genes**		
rpoC1X1 47R	GAA ACT GAT CCA ATT CGG AG	This study
rpoB 3197R	TTA ATC TGG AAG TTC CTC TCA G	This study
rpoB 1977F	TAT GCC GTG GGA AGG TTA	This study
rpoB 2005F	GAT GCG GTA CTT ATT AGT GAG	This study
rpoB 2111R	TTT TCA GGA CCT TGA CTT GTC	This study
rpoB 865F	GCT GCG GAT CAT TTG ATT G	This study
rpoB 988R	CTA GAG CCA ATC CGA ATT G	This study
rpoB 3F	GCT ACG GGA TGG AAA TGA G	This study
rpoCR	GAA ACT GAT CCA ATT CGG AG	This study
rpoC2	CCC AAG CAC TTA TTT GTT GAG	This study
rpoB3Fspin	GCT ACG GGA TGG AAA TGA G	This study
rpo1583Rtob	TTT TCA GGG CCT TGG CTT GTC	This study
rpoB1583RSp	TTT TCA GGA CCT TGA CTT GTC	This study
rpo1679Ftob	TAT CGG GTG GGA GGG TTA C	This study
rpoB1679Fsp	TAT GCC GTG GGA AGG TTA C	This study
rpoB2704Rtob	CCA GAG CCA ATC CGA ATT G	This study
rpoB2791FTob	CTA GAG CCA ATC CGA ATT G	This study
rpoB2704RSp	GCT GCC GAT CAT TTG ATT G	This study
rpoB2791FSpi	GCT GCG GAT CAT TTG ATT G	This study
rpoBF tobacco	GCT CGG GGA TGG AAA TGA G	This study
rpoB-C2	CCC AAG CAC TTA TTT GTT GAG	This study
rpoB-CendR	TTA ATC TGG AAG TTC CTC TCA G	This study
1679Fsubst2	GAT GCG GTA CTT AGT GAG C	This study
***mat*K**		
trnK 3914F	TGG GTT GCT AAC TCA ATG G	Johnson & Soltis 1994
trnK2R	CTA CTC CAT CCG ACT AGT T	Johnson & Soltis 1994
matK-982R	TGA GTC TGT TGA TAC ATT CGG	This study
matK-905F	GAA AAT GCA GGC GAC AAG	This study
psbA-R	CGC GTC TCT CTA AAA TTG CAG TCA T	Johnson & Soltis 1994
matK982R	TGA GTC TGT TGA TAC ATT CGG	This study
matK905F	GAA AAT GCA GGC GAC AAG	This study

matK-4R	GCC AAA GTT CTA GCA CAA G	This study
matK8F	CTT CGA CTT TCT TGT GCT	Steele & Vilgays 1994
psbAR	CGC GTC TCT CTA AAA TTG CAG TCA T	Johnson & Soltis 1994
***rbc*L**		
rbcLR	GAT TTC CTT CCA TAC CTC AC	Panero & Crozier 2003
rbcL650	CAG GTG AAA TCA AAG GGC	Panero & Crozier 2003
rbcL1	ATG TCA CCA CAA ACA GAR ACT AAA GC	Olmstead, 1992
rbcL2	CTT TTA GTA AAA GAT TGG GCC GAG	Olmstead, 1992
rbcL2Rint	TCC ACC AGA CAG ACG TAA CG	This study
***rpl*16**		
rps3F	TCC CCT ACA AAC GAT TCG	This study
R1661	CGT ACC CAT ATT TTT CCA CCA CGA C	Kelchner & Wendel 1996.
F71	GCT ATG CTT AGT GTG TGA CTC GTT G	Kelchner & Wendel 1996.
1516R	CCT TCA TTC TTC CTC TAT GTT G	Kelchner & Wendel 1996.
rpl16 647R	GGT TCG TTC CGC CAT CC	This study
rpl16 1578R	TCC AAG CAG GTT CAA GTG	This study
***trn*T-*trn*L**		
Taberlet a	CAT TAC AAA TGC GAT GCT CT	Taberlet 1991
Taberlet b	TCT ACC GAT TTC GCC ATA TC	Taberlet 1991
***trn*L-*trn*F**		
Taberlet e	GGT TCA AGT CCC TCT ATC CC	Taberlet 1991
Taberlet f	ATT TGA ACT GGT GAC ACG AG	Taberlet 1991
***rpl*20-*rps*12**		
rpl20	TTT GTT CTA CGT CTC CGA GC	Hamilton 1999
5'rps12	GTC GAG GAA CAT GTA CTA GG	Hamilton 1999

Phylogenetic analysis

Alignment and character statistics

Raw nucleotide sequences were proofread and trimmed using Sequencher (v3.1 or 4.1, Gene Codes Corp.) and the assembled 'contig' matrices for each sequencing primer interleaved into genomic units using PAUP* (v. 4.0b10, Swofford, 2000). Coding sequences were aligned as described in Chapter 2. To hypothesize positional homology of non-coding sequences we used ClustalX (Thompson et al., 1997) and adjusted by eye using Seq-App (Gilbert, 1992) using the structural motifs identified for Group II introns as a guide (Kelchner, 2002). Dense taxon sampling facilitated alignment. Gaps were treated as missing data in all analyses. Base composition and nucleotide substitution process statistics were gathered and summarized in Table 3.2. For each locus differences in base composition among taxa were examined for all informative sites using the homogeneity χ^2 test implemented in PAUP*.

Evolutionary model selection

Selection of best-fit models of nucleotide and amino acid substitution from among a set of standard models was accomplished in Modeltest 3.06 (Posada & Crandall, 1998) and evaluated using the Akaike Information Criterion (AIC, Akaike, 1974). The gamma distributions for Bayesian analyses were approximated with six rate categories: two transition rate classes and four transversion rate classes.

Phylogenetic inference

Maximum parsimony (MP) and maximum posterior probability (Bayesian) criteria were used to estimate topology for each locus individually and as a concatenated set. MP tree searches were implemented in PAUP*. As an alternative to searching for an optimal (shortest) tree under MP criteria, Bayesian inference was used to sample possible

trees according to their posterior probability calculated using Bayes' theorem. Bayesian analyses were implemented in MrBayes (v. 3.0b4 either serial or parallel versions, Huelsenbeck & Ronquist, 2001). All data partitions analyzed and discussed here are taxonomically equivalent.

Because of the relatively large number of taxa, MP heuristic searches to find most parsimonious trees were conducted as follows: First, 100 random taxon addition replicates were performed with only 10 trees saved each iteration. Trees saved by this search were then branch-swapped using tree bisection-reconnection (TBR) to check for shorter solutions and to fill out tree space to a limit of 5000 trees at this length. Equally weighted characters were optimized by accelerated transformation (ACCTRAN). Bootstrap resampling (Felsenstein, 1985) with 100 pseudoreplicates was used to evaluate internal clade support.

Bayesian inference was accomplished via a Metropolis-coupled Markov-chain Monte Carlo (MCMCMC; Li et al., 1996; Larget & Simon, 1999; Huelsenbeck & Ronquist, 2001) approximation running four simultaneous MCMC chains: one cold and 3 incrementally "heated", temp=0.5, to facilitate mixing. Chains were run for 10 million cycles. All priors were set according to the preferred GTR model stated above, dirichlet priors for the rate matrix, and uniform priors for the shape and proportion of invariant sites. All runs were started from random trees. Log-likelihoods and trees including branch lengths were sampled every 100 generations. Four independent replicates of each analysis were run and compared for apparent stationarity levels to check that analyses had not been trapped in local optima. Log-likelihood values of sampled trees were plotted against generation number and compared with graphs of replicate runs to determine the initial point at which stationarity was reached (not shown). Trees sampled before stationarity were discarded as burn-in. The mean, standard deviation and 95%

confidence interval of model parameters was calculated for trees sampled after stationarity. Probabilities $\geq$ 95% are considered significant.

RESULTS

BAYESIAN ANALYSES

Combining data slightly increased resolution and support in several clades over the three-gene partition alone (Figs. 3.4, 3.5 vs. 3.6, 3.7). The results are equivalent to those presented in chapter 2. In the majority rule consensus tree of Cactaceae resulting from Bayesian analysis (Figure 3.6) the 15 species of the genus *Pereskia* sampled appear basal within the family. *Pereskia* is not paraphyletic but rather it contains three main lineages splitting sequentially. The Venezuelan species *P. guamacho* is sister to all other species of Cactaceae sampled, followed by a clade of *Pereskia* including the Panamanian species *P. bleo* as sister to the insular, Caribbean species *P. quisqueyana*, *P. portulacifolia* and *P. zinniiflora*. The next clade to split contains most of the species of *Pereskia* sampled and is characterized by having two main clades, one containing the southeastern South American tropical and subtropical species centered about *P. grandifolia* and the other clade contains the Mexican and Andean species of *Pereskia* along with members of subfamily Opuntioideae. The Mesoamerican species *P. lychnidiflora* is sister to the clade containing two lineages, the first clade of which has the Andean members of the genus including *P. aculeata*, *P. horrida*, P. *diaz-romeroana*, and *P. weberiana*. This clade is sister to members of subfamily Opuntioideae. *Maihuenia* of subfamily Maihuenioideae is the next lineage to split followed by the monotypic genus *Blossfeldia* of subfamily Blossfeldioideae. The *rpo*C1 intron is present in *Blossfeldia* as in the Opuntioideae, *Pereskia* and *Maihuenia*. The terminal clade includes most species of the family and is characterized by two major clades representing members of

subfamily Rhipsalidoideae and subfamily Cactoideae respectively. The relationships of subfamily Rhipsalidoideae revealed by this study are equivalent to those outlined in Chapter 2 and will not be discussed further here.

Relationships in subfamily Cactoideae are fully resolved and the clades are supported by posterior probabilities between 95 and 100 percent unless otherwise indicated (Figure 3.6). The Cactoideae constitute a grade with a terminal clade containing two main lineages including members of *Mammillaria* and related genera. The genera *Geohintonia* and *Aztekium* are the basalmost lineage of the Cactoideae. These two genera are sympatric and occur on gypsum or mica shale substrates. The following lineage contains the distinctive genus *Astrophytum* and the genus *Echinocactus*. Like previous lineages, these genera are also found in the deserts of northeastern and central Mexico. *Ferocactus*, *Leuchtenbergia*, *Glandulicactus*, *Stenocactus* and *Thelocactus* are grouped together and constitute a strongly supported clade. The genus *Pediocactus* appears to be an isolated lineage but its position within Cactoideae is equivocal and weakly supported by a posterior probability of only 83%. The next lineage to split contains the genus *Epithelantha,* endemic to the southwestern USA and northern Mexico, as sister to the genera *Gymnocactus*, *Turbinicarpus*, *Ariocarpus* and *Strombocactus*, all essentially endemic to Mexico and characterized by their spineless or weakly spiniferous, tuberculate, disk-shaped habit. The clade

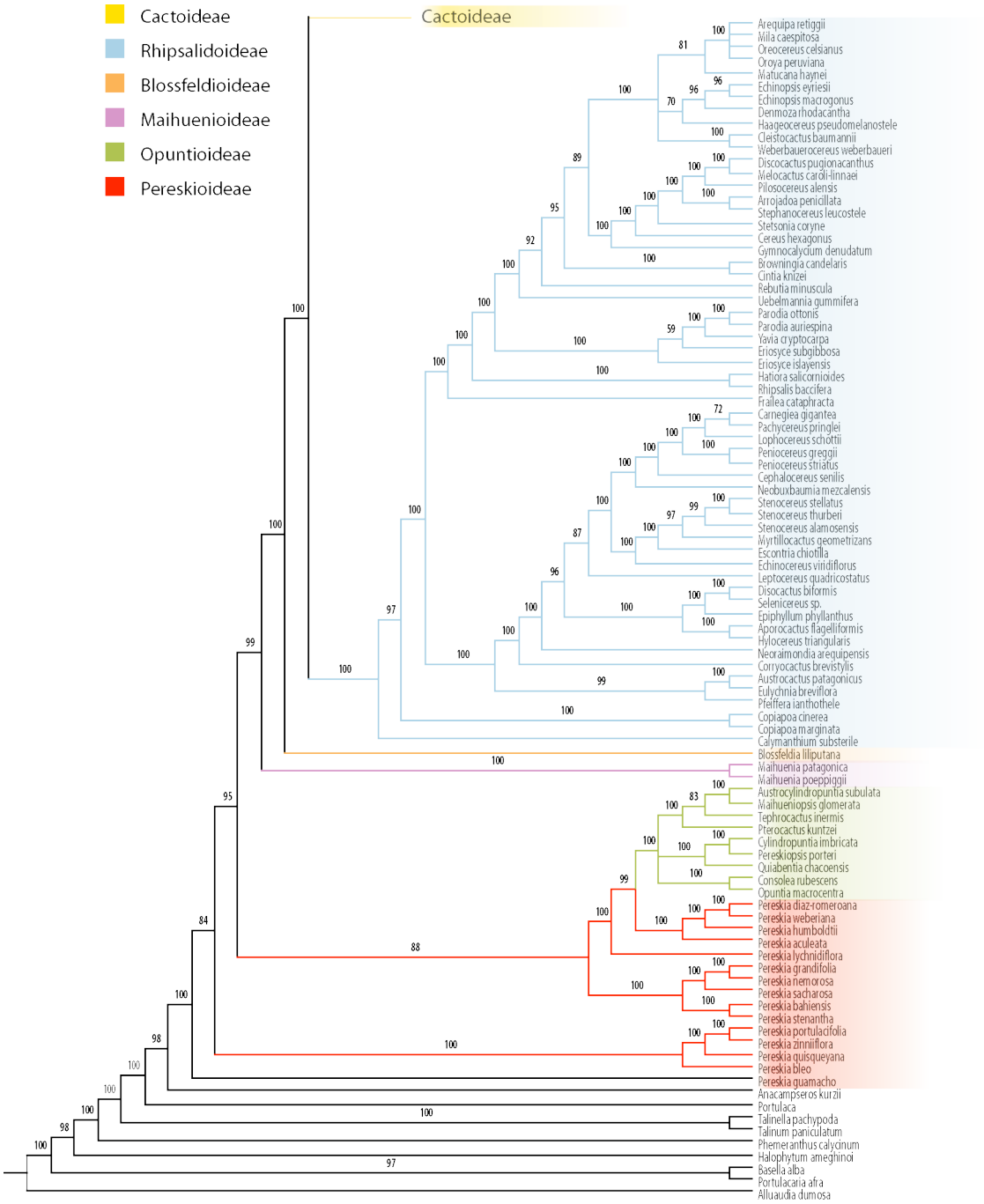

Figure 3.4. Majority rule consensus of trees resulting from Bayesian analyses of the combined data for 157 taxa of Cactaceae. Bayesian posterior probabilities are shown above branches.

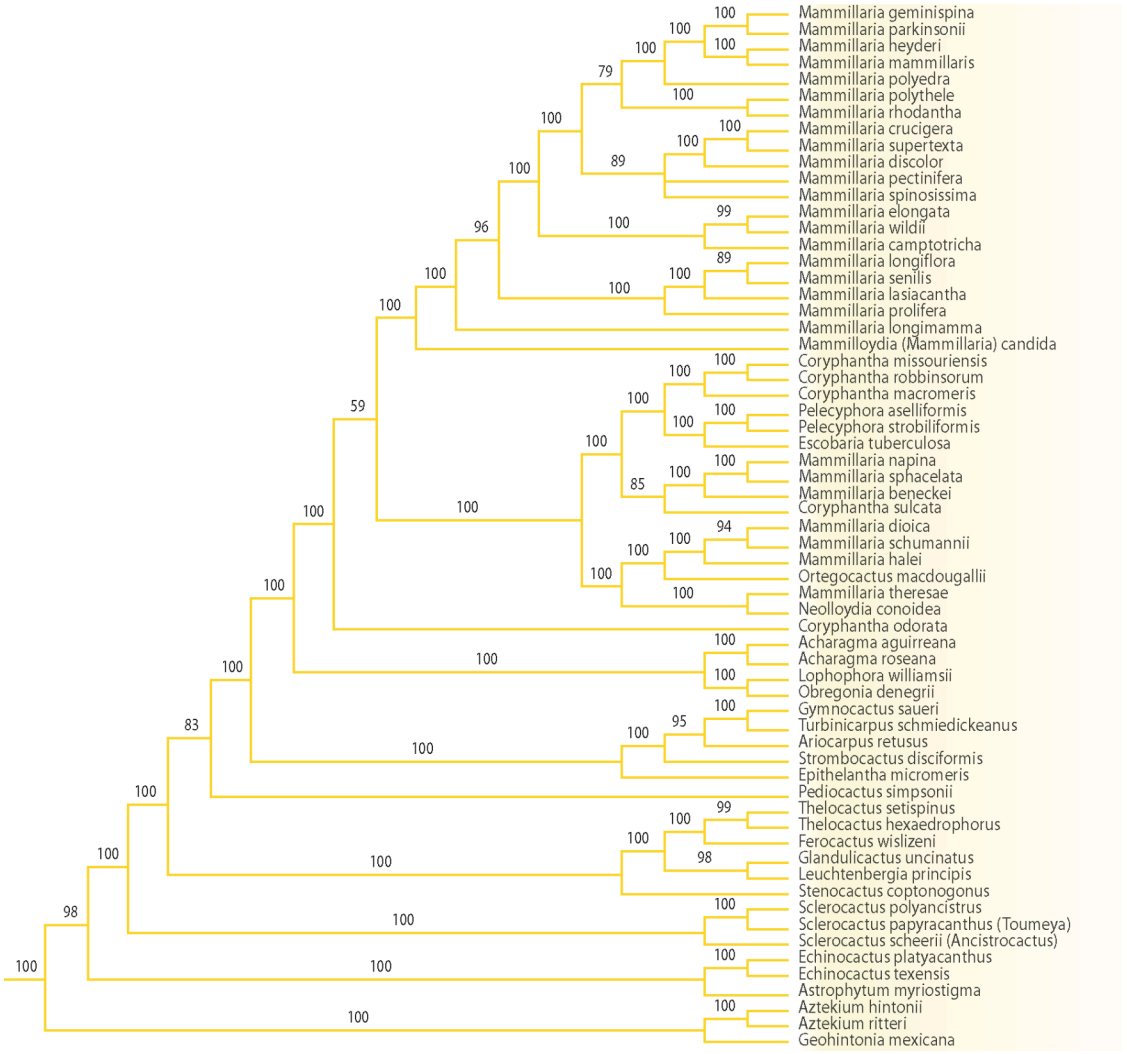

Figure 3.5. Cactoideae cont. Majority rule tree from Bayesian analyses of the combined data for 157 taxa of Cactaceae. Bayesian posterior probabilities are shown above branches.

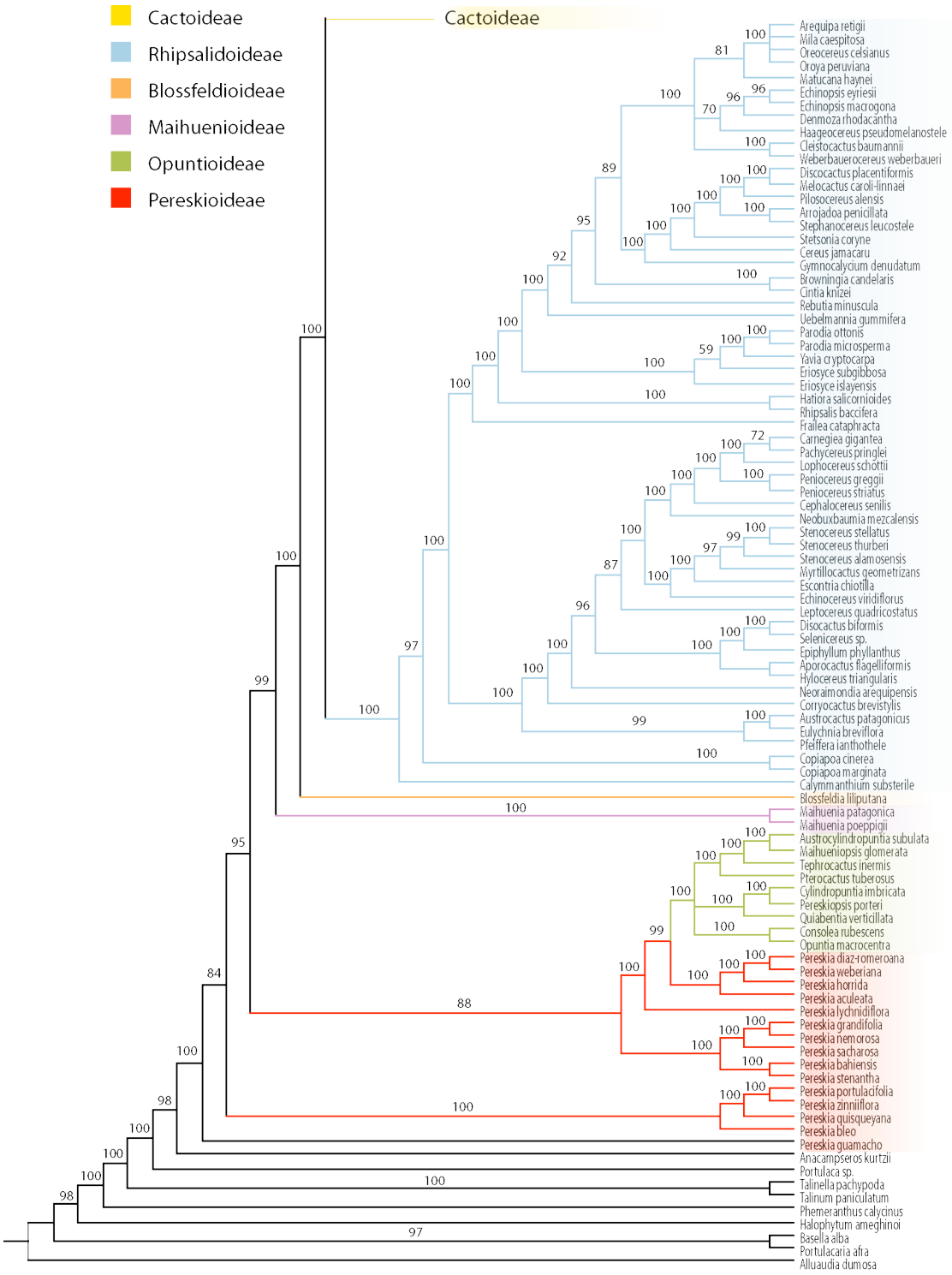

Figure 3.6. Majority rule consensus tree resulting from Bayesian analysis of coding regions *mat*K, *rbc*L and *rpo*B combined.

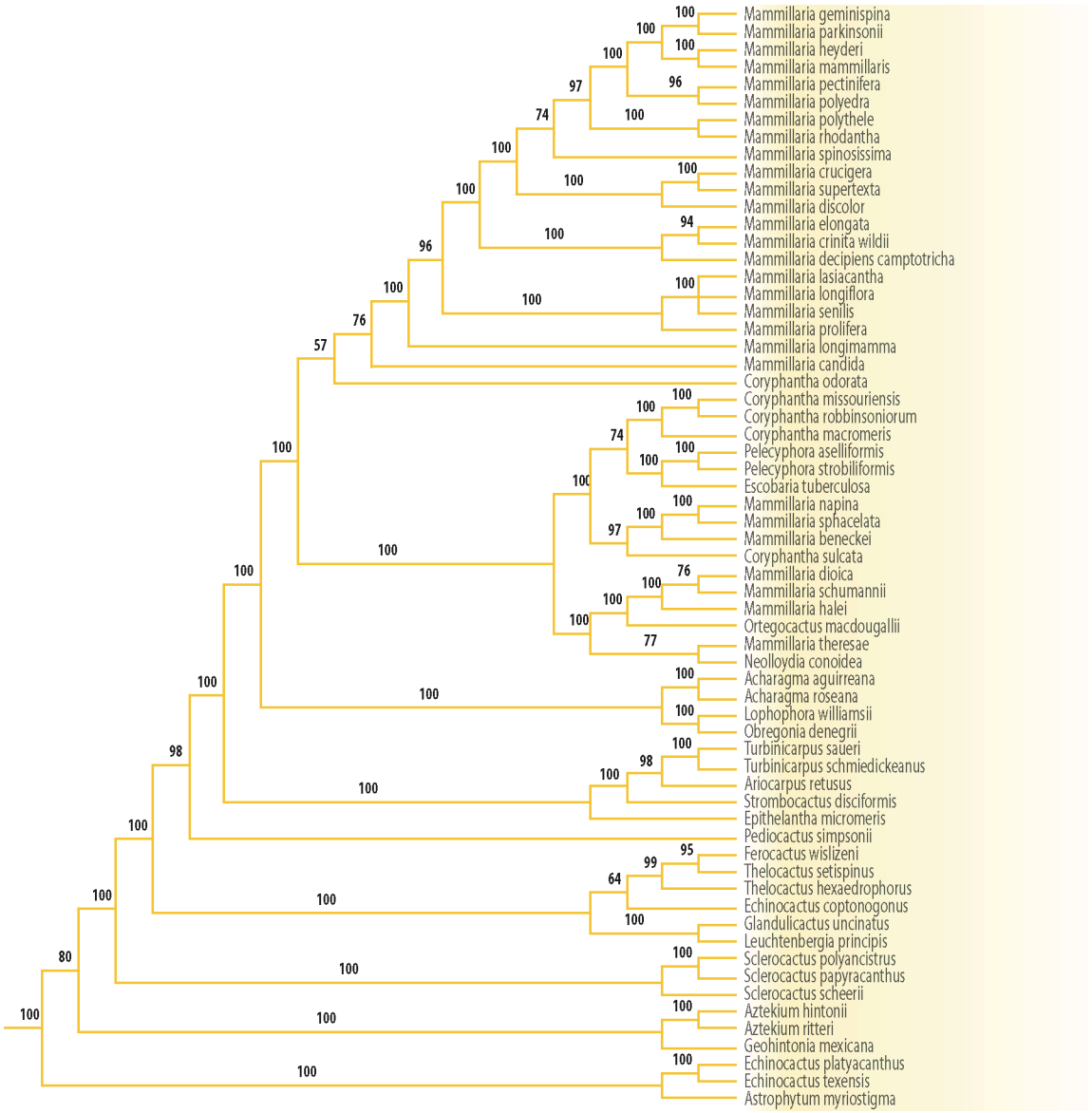

Figure 3.7. Cactoideae, continued from Figure 3.6. Majority rule consensus tree resulting from Bayesian analysis of coding regions *mat*K, *rbc*L and *rpo*B combined.

containing the genera *Acharagma*, *Lophophora* and *Obregonia* are sister to the *Mamillaria*/*Coryphantha* clade which corresponds to the Coryphanthinae. The Coryphanthinae contains three major lineages (all Figures). The monotypic genus *Cumarinia* (*Coryphantha*) *odorata* is sister to two major clades one containing the type species of the genus *Mammillaria*, *Mammillaria mammillaris*, (*Mammillaria* clade) and a *Coryphantha* clade that contains most genera historically associated with *Mammillaria*, in addition to some species of *Mammillaria*. The relationship of *Cumarinia* to these two clades is very weakly supported by a posterior probability of 57%. The *Mammillaria* clade is characterized by the sequential splitting of lineages with *M. candida* or *Mammilloydia candida* most basal followed by another *Mammillaria* segregate at the genus or subgenus level, *Mammillaria* (*Dolichothele*) *longimamma*. The next clades contain an array of species that represent at the basalmost lineages members of *M.* sect. *Hydrochylus* followed by those of *M.* sect. *Subhydrochylus* and with the terminal clades represented by several species of *M.* sect. *Mammillaria*.

The *Coryphantha* clade contains most species of *Mammillaria* traditionally placed in subgenera other than subgenus *Mammillaria* plus members of the genera *Coryphantha*, *Escobaria*, *Neolloydia*, *Ortegocactus*, and *Pelecyphora*. The clade contains two main clades one containing the type species of the genus *Coryphantha*, *C. sulcata* along with the *Mammillaria* species *M. napina*, *M. sphacelata*, and *M. beneckei*, which with the exception of *M. napina,* are all the type species of monotypic genera. The sister clade contains *Escobaria* sister to *Pelecyphora* and collectively sister to *Lepidocoryphantha* and *Neobesseya*. All these taxa are sister to a clade containing the type species of *Mammillaria* subg. *Cochemiea*, *M. halei*, sister to other species of *Mammillaria* also

endemic to the Baja California Peninsula. The diminutive *Mammillaria theresae* of western Mexico is sister to *Neolloydia*.

Unweighted maximum parsimony analyses of the data produced a strict consensus tree with an overall topology similar to the one produced by Bayesian analysis but with less resolution, and with only weak bootstrap support for several clades throughout the tree. The major relationships within Cactoideae resulting from the Bayesian analysis are congruent with those obtained from parsimony analyses. A major difference between the Bayesian and parsimony analyses is in the lack of resolution in the terminal *Mammillaria* clade. The sister-relationship of the clade containing *M. polythele* and *M. rhodantha* with the clade containing members of *M.* sect. *Mammillaria* is not supported, and the clade containing *M. spinosissima* and *M. pectinifera* also collapses in the MP analyses strict consensus tree. In the Bayesian analyses of the coding data, *M. pectinifera* is sister to *M. polyedra*. The sister relationship of *Mammilloydia candida* and the lineage containing *Mammillaria longimamma* sister to the rest of the mammillarias sampled is supported in the parsimony analyses. The topology of the *Coryphantha* clade is identical in both analyses but some of the clades do not have bootstrap support in the parsimony tree (Figs. 3.8, 3.9).

Maximum Parsimony analyses of the coding loci data set for all 157 taxa produced a less resolved, topologically congruent tree and will not be discussed further.

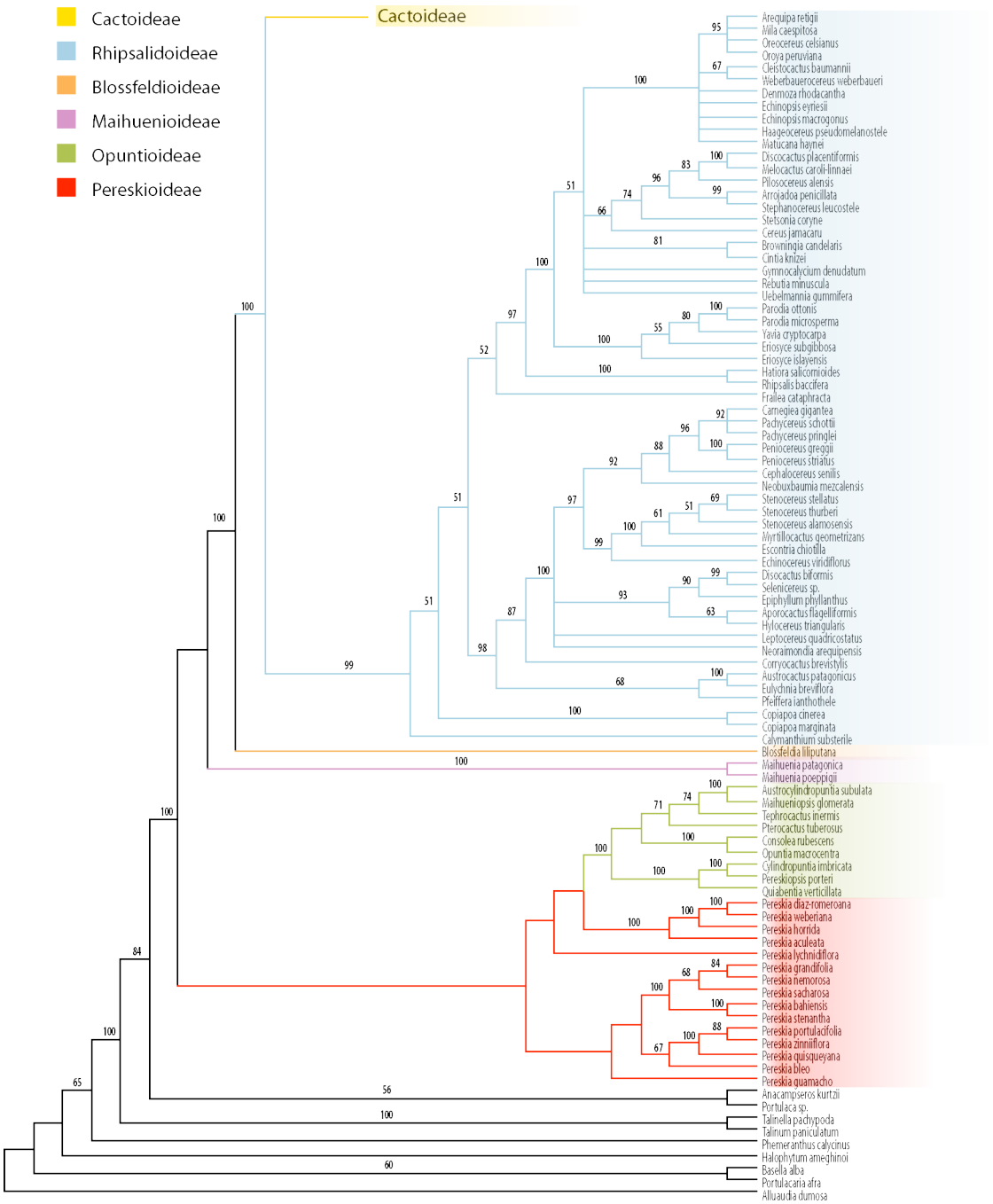

Figure 3.8. Strict consensus of 85,008 trees found in full heuristic MP 1000 random addition sequence replicates, maximum parsimony search using TBR branch swapping. Numbers above branches indicate fast bootstrap values greater than 50%. Number of characters 13,165, tree length 10311, CI = 0.4515, RI = 0.5485.

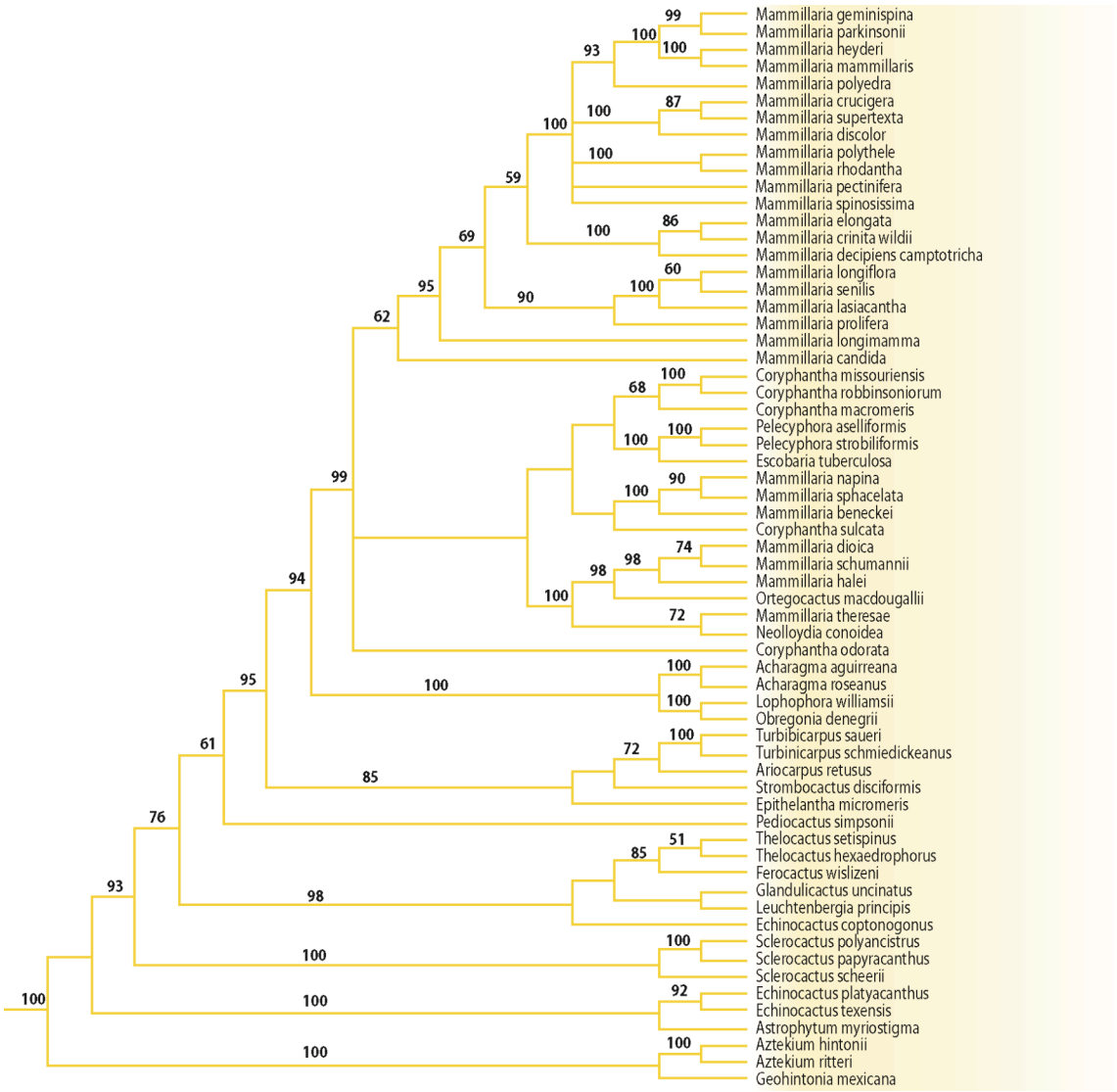

Figure 3.9. Cactoideae, continued from Fig. 3.8. Strict consensus of 85,008 trees found in full heuristic 1000 random addition sequence replicates, maximum parsimony search using TBR branch swapping. Numbers above branches indicate fast bootstrap values greater than 50%. Number of characters 13,165, tree length 10311, CI = 0.4515, RI = 0.5485.

DISCUSSION

Relationships among the major clades of Cactaceae

The focus of this paper is on phylogenetic relationships of the genus *Mammillaria* and related genera in the subfamily Cactoideae (sensu Crozier 2004). However, this study was designed so that results might also be useful in the creation of a new phylogenetic classification for the family Cactaceae outlined in Chapter 4. Every effort was made to obtain type species of each genus included in this study so that pronouncements related to basic taxonomic decisions in classification could be made. Several interesting results in other subfamilies of the Cactaceae are discussed briefly here in light of their importance in the elucidation of phylogenetic relationships within the family.

The monophyly of the Cactaceae is well supported by my data, congruent with morphological studies (Gibson & Nobel 1986) and previous molecular studies (Hershkovitz & Zimmer, 1997; Nyffeler, 2002). Nine taxa, including several representatives of the Portulacaceae, Basellaceae, Halophytaceae and Didieraceae, were included in the analyses and the monophyly of the Cactaceae is strongly supported with 100% bootstrap and Bayesian probability. The Cactaceae is characterized by felted short shoots termed areoles from which flowers, spines or leaves arise. The inferior ovary of Cactaceae is enclosed by stem tissue that in most cases extends to the floral perianth parts. My data also show that the Portulacaceae is apparently not monophyletic but rather a grade sequentially splitting lineages leading to the Cactaceae. This result is not unusual and has been reported extensively in the literature (see Stevens, 2001 onwards for references and commentary). Results of Bayesian and parsimony analyses support the recognition of at least 6 subfamilies in the Cactaceae.

My studies show that the genus *Pereskia* is a paraphyletic assemblage at the base of the family composed of either 3 or 4 different lineages. The subfamily Opuntioideae appears to be derived from the lineage of *Pereskia* containing the Andean species of the genus centered about *P. horrida*.

The southern Andean genus *Maihuenia* containing two species endemic to cold deserts or grasslands at the base of the Andes, has sometimes been viewed as a connecting link between Opuntioideae and Pereskioideae. *Maihuenia*, the sole genus of the Maihuenioideae, resembles, albeit superficially, species of the sympatric Opuntioideae genus *Tephrocactus* with the difference that *Maihuenia* has persistent leaves and lacks glochids and its seeds lack a bony aril, a characteristic of Opuntioideae. The *Maihuenia* clade which diverges above the Pereskioideae and Opuntioideae lineages is sister to the rest of the Cactaceae.

The next lineage to split is that of the monotypic genus *Blossfeldia*, the only member of the Blossfeldioideae. *Blossfeldia liliputana* is endemic to the mid-elevation, dry, shale hillsides on the eastern side of the Andes in northern Argentina and southern Bolivia. *Blossfeldia* is the smallest cactus known and contains stomata only in its areolar pits. The morphological gap between this highly reduced and specialized cactus and the rest of the family could be interpreted as the result of specialization or accelerated evolution without persistent intermediate steps.

Parsimony and Bayesian analyses provide strong support for the sister relationship of Cactoideae and Rhipsalidoideae. Approximately 75% of the species of Cactaceae belong to this clade (Barthlott & Hunt 1993, Hunt 1999, Anderson 2001). Subfamily Rhipsalidoideae includes most of the columnar cacti of North and South America and the globular cacti of South America. They are characterized by the presence of areoles and spines on the abaxial surfaces of the flowers of most of its

species. The relationships of the Rhipsalidoideae were recently explored by Nyfeller (2002) who produced a relatively well-resolved phylogeny for the group in which he identified several of the major clades of the subfamily. My studies support some of his conclusions and provide additiona; evidence for novel relationships not recorded before in the literature and these are incorporated into a new classification of the family outlined in Chapter 4.

The monotypic arboreal genus *Calymanthium* from northern Peru is sister to all other Rhipsalidoideae. This genus is distinctive in Cactaceae because of its floral morphology in which the flower breaks through the pericarpel structure. The fruit produced by this flower is probably the largest in the family. The genus *Copiapoa* of the Atacama Desert of northern Chile is also an isolated lineage and next lineage to split. *Copiapoa* is characterized by its glabrous, mostly yellow flowers, apical trichomes, and central, apical flowering. Most species thrive in the foggy, cold desert of northern Chile. Rhipsalidoideae diverges in 2 main clades above the *Copiapoa* lineage each clade has one lineage containing small, globular or weakly columnar species sister to clades containing epiphytic and massive, arboreal columnar species of cacti in North and South America, clearly supporting parallel origins for these features. A more detailed discussion of the systematics and classification of the Rhipsalidoideae is provided in Chapters 2 and 4.

Subfamily Cactoideae

The phylogenetic relationships of subfamily Cactoideae have received increased attention in the past few years with several studies based on molecular data aimed at understanding the phylogenetic relationships of this mostly North American group Butterworth et al. (2002), Butterworth & Wallace (2004, etc). The latter studies have been helpful in identifying the major clades of the Cactoideae but poorly resolved trees

and weak clade support has limited the value of their conclusions. My studies also based on chloroplast DNA data provide for the first time a strong phylogenetic hypothesis of relationship for the Cactoideae and shed light on the taxonomic limits of the genus *Mammillaria*.

The Cactoideae are mostly globular cacti with areoles arranged spirally or in smooth or tuberculate ribs. The subfamily is further characterized by its seeds which have a disjunct, rarely fused, hilum and micropylar areas (Barthlott & Voit, 1979; Crozier, 2004), floral pericarpels without areoles or spines, and a mostly North American distribution. In addition, the monophyly of the subfamily has been confirmed by several molecular studies ranging from the presence or absence of a particular piece of chloroplast DNA (Wallace & Cota, 1996; Applequist & Wallace, 2002) to comparative studies of sequences of the *mat*K gene for the whole family (Nyfeller, 2002).

Butterworth et al. (2002) provided the first molecular phylogeny for all the genera recognized by recent authors as belonging to Cactoideae (Barthlott & Hunt, 1993; Anderson, 2001). Although the monophyly of the subfamily is difficult to ascertain given their limited use of outgroup taxa, nevertheless their study represents the first hypothesis of generic relationship within Cactoideae based on molecular studies. Their results show that the evolution of the Cactoideae has proceeded by the sequential splitting of lineages either containing a few genera or sometimes a single genus, with the more derived, recent lineages belonging to members of *Mammillaria* and related genera. For the most part, their phylogenetic conclusions are supported by our results, except for three important differences: the placement of the *Acharagma*, *Lophophora*, *Obregonia* clade and the clades containing the genera *Ferocactus* and *Stenocactus*.

Bayesian and maximum parsimony analyses of the data strongly support the monophyly of subfamily Cactoideae (Figs. 3.5, 3.7, 3.9). *Aztekium* and *Geohintonia* are

sister to the rest of the subfamily. The genus *Aztekium* is unusual among Cactoideae because of their compound ribs which, like *Blossfeldia*, tend to have stomata sunken in the pits or grooves of ribs. The two species of *Aztekium* and the monotypic *Geohintonia* are narrow endemics to the eastern flanks of the Sierra Madre Oriental of central and southern Nuevo León, Mexico. *Astrophytum* and *Echinocactus* comprise the next lineage. These two genera have a more extensive, parapatric distribution in the eastern half of the Chihuahuan desert. Both genera share acicular, spine-tipped floral scales, the scales in *Echinocactus* are pubescent. *Astrophytum* and *Echinocactus* have a long taxonomic history, the former as a distinctive genus because of their star-shaped habit and the peculiar scales covering their body, and *Echinocactus* as the repository of most species of barrel-like cacti with areoles arranged along ribs. *Echinocactus* has been redefined to include most cacti with a dense tuft of trichomes on their growing apices from which flowers protrude. The genus appears not to be monophyletic as *Echinocactus grusonii* (not sampled in this study) is more closely related to *Ferocactus* than to the clade containing the type of the genus, *Echinocactus platyacanthus* (Cota & Wallace, 1997; Butterworth & et al., 2002). In our studies, *Echinocactus texensis*, the basis of the genus *Homalocephala* Britton & Rose is sister to *Echinocactus platyacanthus*. The genus was considered distinct because of its flattened body. We agree with previous workers who consider *Homalocephala* a synonym of *Echinocactus* (Bravo-Hollis & Sánchez-Mejorada, 1991; Ferguson 1992; Barthlott & Hunt, 1993).

The genus *Sclerocactus*, along with *Pediocactus*, is distinctive among other Cactoideae because its distribution is mostly confined to the western deserts of the United States; the genus barely reaches Mexico. *Sclerocactus* was named by Britton & Rose (1922) to include a series of species that encompass a distinctive combination of characters including tuberculate seeds, thick ribs, and glabrous floral scales. Several

species of *Sclerocactus* have been segregated as different genera based on characters of the spines. *Toumeya* was considered different by Britton & Rose because of its papery, flat spines whereas *Ancistrocactus* was deemed distinctive at the genus level because of its hooked spines. Our studies show that *Sclerocactus* and its segregates are monophyletic. As reported by Porter et al. (2000) and Butterworth et al. (2002) my results also confirm that *Pediocactus* is not closely related to *Sclerocactus*. My results also support the recognition of the genus *Glandulicactus* as a member of the *Ferocactus* clade, as advanced by Ferguson (1991) based on morphological characteristics, and Butterworth et al. (2002) based on molecular data. The genus *Echinomastus*, not sampled here, was placed in the synonymy of *Sclerocactus* by Hunt (1999) despite its ribbed rather than tubeculate stems. Based on our results, the *Sclerocactus* lineage represents the first transition from ribbed to tuberculate stems in the Cactoideae.

The *Ferocactus* clade contains the genera *Ferocactus* as sister to *Thelocactus* and collectively sister to *Glandulicactus* and *Leuchtenbergia*; *Stenocactus* is sister to them. The position of this clade as the next to split above the *Sclerocactus* clade is not congruent with the results of Butterworth et al. (2002) who place this clade sister to their mammilloid or *Mammillaria* clade. The position of this clade is strongly supported in the Bayesian analyses with a posterior probability of 100% and weakly supported in the parsimony analyses with a bootstrap value of 76. The species of this clade are characterized by having glabrous pericarpel scales, pitted seeds, superficially similar to those of *Mammillaria*; because of this they are historically considered to be the sister taxon of the *Mammillaria* clade (Britton & Rose 1922-1923; Zimmerman, 1985). As explained by Butterworth et al. (2002) the *Ferocactus* clade contains taxa with mostly ribbed stems, although the genus *Leuchtenbergia* and certain species of *Thelocactus* exhibit tuberculate stems. All the lineages splitting above the *Ferocactus* clade have

tuberculate stems. Therefore, the evolution of tuberculate stems has occurred at least four times in the evolution of the Cactoideae, two of these independently in the *Ferocactus* lineage.

The genus *Pediocactus* contains approximately six species distributed in the western United States. The position of this clade is weakly supported in the Bayesian analyses with a posterior probability of 83%, and weakly supported in the parsimony analyses with a bootstrap value of 61. My analysis cannot state confidently the phylogenetic relationships of this genus. Additional sampling including other species of the genus is necessary.

The ATEP clade of Butterworth et al. 2002 contains the genera *Ariocarpus*, *Turbinicarpus*, *Epithelantha*, and *Pediocactus*. Our studies, albeit weakly, support the exclusion of the genus *Pediocactus* from this clade and the inclusion of the genus *Strombocactus*. Our ATES clade contains the genera *Ariocarpus*, *Turbinicarpus*, *Epithelantha,* and *Strombocactus*. *Strombocactus* was named by Britton & Rose to accommodate an unusual species originally described in *Mammillaria*. They considered this taxon distinctive because of its conical stems and short tubercles. *Strombocactus* is a narrow endemic to the driest areas of the state of Hidalgo and Queretaro in central Mexico and like *Aztekium* it is usually found growing on vertical cliffs indeed, Buxbaum (1958) believed the genus to be related to *Aztekium*. Our studies, however, show that *Strombocactus* is sister to *Turbinicarpus* and *Ariocarpus*. *Strombocactus* shares with *Turbinicarpus* and *Ariocarpus* a tendency to have soft-spinned or spineless tubercles. The genus *Ariocarpus*, like genera of the *Mammillaria* clade, has dimorphic areoles with spines and flowers produced in different areas of the tubercle.

The *Acharagma*, *Lophophora*, *Obregonia* clade is sister to the *Mammillaria* clade. Zimmerman (1985) considered *Acharagma* a primitive *Mammillaria* and likely

derived from the *Ferocactus* lineage. According to Zimmerman (1985) the foveolate seeds of *Acharagma* show a relationship not only to *Mammillaria* but also to *Ferocactus*, and the other genera having this type of seeds including *Escobaria,* and *Ortegocactus*. The two species of *Acharagma* were originally placed by Glass and Foster (1970) in the genus *Gymnocactus* and later transferred by Anderson and Ralston (1978) to *Turbinicarpus*. Taylor (1986) placed the two species in the genus *Escobaria* in his section *Acharagma*. Species of section *Acharagma* lacked the areolar groove typical of the genus *Escobaria* and the flowers are borne distally on the tubercle and close to the areole as opposed to centrally like in most species of *Escobaria* and related genera. Glass (1998) elevated *Escobaria* sect. *Acharagma* to generic rank. *Acharagma*, because of its seed morphology was viewed as closely related to *Mammillaria*, *Escobaria*, *Ortegocactus* and *Ferocactus*. The close relationship of *Acharagma* to *Lophophora* or *Obregonia* has never been discussed or even hypothesized in the literature. *Lophophora* because of its globose bluish stems, spineless areoles with a penicillate tuft of trichomes, and peculiar phytochemistry has always been viewed as a distinctive genus in Cactoideae. Its fruits, like those of *Obregonia*, are glabrous. *Obregonia* shares with *Lophophora* a similar areole structure, seed morphology, floral position, and fruit size (Anderson, 1967).

***Mammillaria* and related genera.**

The primary goal of this study was to understand the taxonomic limits of *Mammillaria* and its relationships to those genera having areolar grooves as exemplified by *Coryphantha*, *Escobaria* and *Neolloydia*. Other authors (Butterworth et al., 2002; Butterworth & Wallace, 2004) have published molecular studies aimed at answering the questions posed here. Their studies, however, failed to resolve the taxonomic limits of *Mammillaria*, but hey did identify the *Mammillaria* clade as a derived lineage of the

Cactoideae. Ironically, their molecular phylogeny of *Mammillaria* is a less detailed hypothesis of relationships than those based on morphological studies (Hunt, 1981; Lüthy, 1995, 2001) (Fig. 2). Our study incorporates the type species of all the infrageneric categories recognized by Hunt, and a great majority of those recognized by Lüthy. In addition, the type species of all the genera historically linked to *Mammillaria* have been included in the study. The aim is to answer the question, What is a *Mammillaria*?

The classification of the genus *Mammillaria* has gained tremendous stability through the work of Hunt (1971, 1981) and Lüthy (1995, 2001). Their classifications rely on multiple morphological characters whose states are polarized by comparison with presumed outgroup taxa. Milky sap is absent from any other genus related to *Mammillaria* and is generally viewed as a derived condition in the genus. Those species lacking milky sap are similar to the outgroups and therefore, are viewed as having the primitive condition, while the most derived species of *M*. subg. *Mammillaria*, sect. *Mammillaria* have milky sap (Hunt, 1981; Lüthy, 1995, 2001). Mammillarias with colored watery sap are intermediate between the watery (clear) and milky sap species in the transition series. Sap characteristics along with seed characters such as the presence or absence of perisperm, degree of embryo curvature, testa morphology and seed coat color, provide the main characters for infrageneric classification.

Mammillaria has always been viewed as a genus with tuberculate stems having dimorphic areoles without any connecting groove. The spiniferous areole is at the tip of the tubercle, whereas the floral areole is axillary. Flowering in *Mammillaria* is invariably below the shoot apex forming a ring of flowers, whereas the sister taxa tend to have their flowers proximal to the center of the stem. As discussed by Zimmerman (1985), this is not a reliable characteristic for the genus because many intermediate conditions bridge

Mammillaria to its sister genera. *Coryphantha* and related genera have an areolar groove along their tubercles. The grove can be complete or partial and this characteristic has not passed unnoticed in the creation of genera segregated from, or infrageneric taxa in, *Coryphantha* or its closely related genus *Escobaria*.

Based on the molecular evidence presented here, the genus *Mammillaria* as currently defined is not monophyletic. This result is in agreement with other molecular studies of Cactoideae and *Mammillaria* (Butterworth et al., 2002, Butterworth & Wallace, 2004). Some species of *Mammillaria*, notably those considered by Lüthy (2001) as primitive in the genus, and those of *M.* subg. *Oehmea* and *Cochemiea*, are nested within the branch sister to *Mammillaria* that contains *Coryphantha*, *Escobaria,* and other monotypic genera segregated from the latter two (Fig 3.5, 3.7, 3.9). Comparing our results with the classifications of Hunt (1981) and Lüthy (1995, 2001) it is clear that their classifications are in agreement with our results in regards to the nominal subgenus. Both authors circumscribe a monophyletic *M.* subg. *Mammillaria*, the milky sap mammillarias, whereas the other sections of subgenus *Mammillaria* as circumscribed by them are paraphyletic (Fig. 3.10). *Mammillaria senilis*, considered by both authors to be a distinct subgenus of *Mamillaria* (*M.* subg. *Mamillopsis*) because of its red, slightly zygomorphic flowers, is sister to *M. longiflora* another species of the state of Durango with long and showy pink flowers. These results support the inclusion of *M. senilis* within subgenus *Mammillaria* and place in its synonymy *M.* sect. *Krainzia* as delimited by Lüthy (2001). The phylogenetic position of *Mammilloydia* (*Mammillaria*) *candida* sister to the *Mammillaria* clade allows for the recognition of this species at the generic rank, distinctive from *Mammillaria* because of its smooth seeds. A similar argument can be used to recognize *Dolichothele* to accommodate *Mammillaria longimamma* and related species. *Dolichothele* was named by Schumann (1899) as a subgenus of

Mammillaria and elevated to genus by Britton & Rose (1923). Later workers believed *M. longimamma*, the type species, and related taxa to be distinctive within *Mammillaria* because of their elongated tubercles and large, yellow flowers with a solid tube. *Dolichothele* occupies a basal position within the *Mammillaria* clade, the next lineage to split after *Mammilloydia*. We have chosen to maintain *Dolichothele* as a subgenus of *Mammillaria* given that its seeds are pitted like in the rest of the species of *Mammillaria*. As is the case for *Mammilloydia*, recognition of *Dolichothele* as currently understood is a matter of taste.

The clade sister to the *Mammillaria* + *Mammilloydia* clade contains the genera *Coryphantha*, *Escobaria*, *Ortegocactus*, *Neolloydia* and a few species of *Mammillaria* (for discussion purposes identified here as the *Coryphantha* clade). The morphology, systematics and nomenclatural history of *Coryphantha* were extensively documented by Zimmerman (1985) in his partial revision of the genus *Coryphantha*. He considered *Coryphantha* in its broadest sense, that is, including the genera *Acharagma*, *Escobaria*, *Lepidocoryphantha*, and *Neobesseya*. He noted the distinctiveness of *Cumarinia* (*Coryphantha*) *odorata* that in our study is revealed as either sister to the *Mammillaria*/*Coryphantha* clade (weakly; Figure 3.5) or sister to the Mammillaria clade (Figure 3.7). *Cumarinia* was segregated from *Coryphantha* by Buxbaum (1951a) because of its deep red fruits (greenish in *Coryphantha*, but see Zimmerman, 1985), lack of perisperm, and hooked, central spines. Because of its position in our tree and its distinctive morphology, *Cumarinia* is neither a *Coryphantha* nor an *Escobaria* but rather a distinctive lineage worthy of recognition at generic rank.

The *Coryphantha* clade has two strongly supported lineages that each include several species of *Mammillaria*. One clade contains the other four species of *Coryphantha* sampled, the type species of *Escobaria*, the two species of the genus

Pelecyphora, and three species of *Mammillaria*. The other clade contains *Neolloydia*, *Ortegocactus* and four species of *Mammillaria*. Bayesian analyses provide strong support for all the clades within the *Coryphantha* clade whereas parsimony analyses show weak to strong support for several of the lineages of this clade (Figs. 3.5, 3.7, 3.9). The type species of *Coryphantha*, *C. sulcata* is sister to the clade containing three species of *Mammillaria*, *M. napina*, *M. sphacelata*, and *M. beneckei*. This relationship is weakly supported in the Bayesian analyses (85%) and has no bootstrap support above 50% in the parsimony analysis. *Mammillaria beneckei* is the type of *M.* subg. *Oehmea*. *Mammillaria beneckei* is distinctive within *Mammillaria* because of its large, foveolate, rugose seeds and yellow flowers with solid tubes. Our study supports the recognition of the genus *Oehmea*. Sister to *Ohmea* is *Mammillaria napina* and *M. sphacelata,* species placed by Lüthy (1995, 2001) and Hunt (1981) in different sections of *Mammillaria* (Fig. 3.10). The two species are essentially sympatric with *M. napina,* growing in the cooler grasslands at the northern rim of the Valley of Tehuacán in central Mexico, and *M. sphacelata* found nearby in an area just barely below these grasslands where the canyonlands meet the desert. *Mammillaria napina* and *M. sphacelata* are very different morphologically with *M. napina* having a globose, essentially sunken stem producing large pink flowers that are reminiscent of species of northern Mexico centered about *M. longiflora*. *Mammillaria sphacelata* is a caespitose species with long, yellowish-green stems that produce red-purple flowers. *Mammillaria sphacelata* is the type of *M.* series *Sphacelatae* of both Lüthy and Hunt, whereas *M. napina* is placed in *M.* series *Longiflorae* of *M.* sect. *Krainzia* (Lüthy, 1995) or sect. *Hydrochylus* Hunt (1981). Doweld (2000) recently named the genus *Escobariopsis* to accommodate a series of *Mammillaria* species that, according to the results of this molecular study, have little in common. *Escobariopsis* should be redefined to include only the type species,

Escobariopsis (*Mammillaria*) *sphacelata*. Recognition of *Escobariopsis* necessitates the creation of a new genus to accommodate *Mammillaria napina*. We refrain from doing this however, because more taxon sampling is needed to understand the relationship of these species.

The two species of the genus *Pelecyphora* are sister to *Escobaria* and collectively sister to the other three species of *Coryphantha* sampled for this study. *Coryphantha missouriensis* was segregated as the type of *Neobesseya* by Britton & Rose (1923) on the basis of its red fruits and black seeds. Bravo-Hollis & Sánchez-Mejorada (1991) expanded the concept of the genus by including the monotypic genus *Ortegocactus* of southern Mexico within *Neobesseya*. Our results support the recognition of *Neobesseya* without the inclusion of *Ortegocactus*. *Ortegocactus* was described by Alexander (1961) on the premise that the genus represented a link between the *Coryphantha*/*Mammillaria* lineage and *Echinocactus*. This statement was based on the apparent fact that the dry, pubescent fruits of *Ortegocactus* were similar to those of the *Echinocactus* clade, whereas the vegetative features of the plant were similar to *Mammillaria*. Zimmerman (1985), noted that the fruit of *Ortegocactus* is glabrous as in all mammillarias and coryphanthas. *Ortegocactus* is sister to the branch containing members of *Mammillaria* subgenus *Cochemiea* as circumscribed by Lüthy (2001). The smooth, rounded tubercles of *Ortegocactus* and its black and white spines are reminiscent of those of *M. schumannii* of subgenus *Cochemiea*.

Mammillaria subgenus *Cochemiea* has always been regarded as a distinctive group within *Mammillaria*, because of its large, red, zygomorphic flowers. Britton & Rose (1923) recognized *Cochemiea* at generic level whereas Hunt (1981) and Lüthy (2001) considered *Cochemiea* a subgenus of *Mammillaria*. Our studies show that *Cochemiea* should be recognized as a distinctive genus of the *Coryphantha* clade. As

circumscribed by Lüthy (2001), the genus also includes species that lack the ornithophilous syndrome of red zygomorphic flowers such as occurs in *M. schumannii* and *M. dioica*. Most species of the genus are endemic to Baja California and adjacent areas of the Sonoran desert.

Neolloydia is sister to *Mammillaria theresae* and collectively sister to *Ortegocactus* and *Cochemiea* (Fig. 3.10). The relationship of *Mammillaria theresae* to *Neolloydia* was not expected as the two species are quite different in most aspects of their morphology. *Neolloydia* has verrucose seeds and grooved areoles, whereas *M. theresae* has pitted seeds and smooth tubercles. *Mammillaria theresae* is a narrow endemic of the dry interior ranges of the state of Durango in northern Mexico. The plant is very small and normally allied to the group of mammillarias with soft, plumose spines such as *M. saboae* Glass and *M. sanchez-mejoradae* Gonzalez. Because of its large flowers on long tubes it has been allied to *Mammillaria longiflora* a plant that is sympatric with *Mammillaria theresae*.

We have provided evidence that the genus *Mammillaria* is not monophyletic as currently circumscribed. To maintain a monophyletic *Mammillaria* necessitates inclusion of the genera *Coryphantha*, *Escobaria*, *Ortegocactus*, and *Neolloydia* in its synonymy. A larger genus *Mammillaria* would include approximately from 250-300 species. This solution would not change the fact that certain species of *Mammillaria* are more closely related to species that have smooth seeds and grooved tubercles such as those of *Coryphantha* and *Escobaria*. In addition, our studies also reveal that the genus *Coryphantha,* as presently understood, has at least four different lineages that can be recognized as genera including *Coryphantha*, *Cumarinia*, *Lepidocoryphantha* and *Neobesseya*. The parallel evolution of key morphological features routinely used in the classification of this species has led to the difficult taxonomy we have today. We

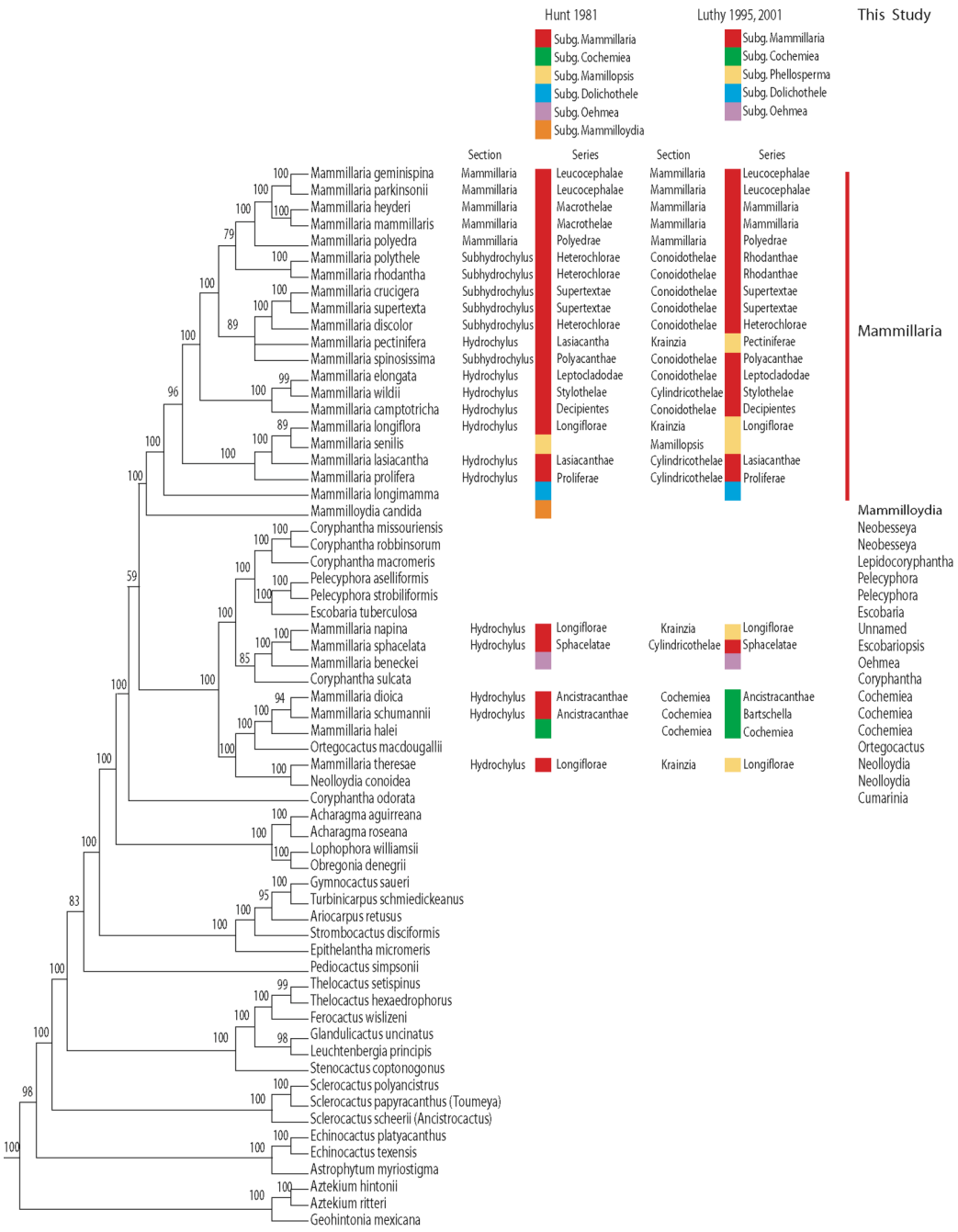

Figure 3.10. Summary of *Mammillaria* classifications: Hunt (1981), Luthy (1995/2001) and proposed new classification (this paper). Majority rule tree summarizing Bayesian post burn-in trees. Posterior probablilities above branches.

propose a smaller genus *Mammillaria* including only most of the species belonging to *M.* subg. *Mammillaria* as circumscribed by Hunt (1981) and Lüthy (1995), and the recognition of the genera *Cochemiea*, *Coryphantha*, *Cumarinia*, *Escobaria*, *Escobariopsis*, *Lepidocoryphantha*, *Neobesseya*, *Neolloydia*, *Oehmea*, *Ortegocactus*, and *Pelecyphora*. Given that some of the relationships revealed by the molecular studies were not expected if morphological similarity were used as an indicator of relatedness, extensive sampling of those species in subgenus *Cochemiea* and series *Longiflorae* group is needed. Future studies will focus on elucidating the phylogenetic relationships of all the species of the genus *Mammillaria* and related genera using sequence data of coding regions of the chloroplast DNA as well as nuclear markers.

Chapter 4: A revised classification of Cactaceae Juss. based on chloroplast DNA analyses

INTRODUCTION

The Cactaceae is estimated to comprise at least 1500 species in 105 genera (Hunt, 1999). The taxa are succulent and noteworthy for their morphological and anatomical adaptations to arid habitats. The family is primarily distributed in the New World. A single species, *Rhipsalis baccifera,* extends to the Old World in southern Africa, Madagascar, and Sri Lanka perhaps as the result of long distance avian dispersal of its fruit, a sticky berry. Cacti are most common in dry habitats between the 35th latitudes, ranging from British Columbia in Canada to Patagonia near the Straits of Magellan, but are absent from the Amazon region (Barthlott & Hunt, 1993; Taylor, 1997). Three genera occur in the Galápagos Islands in the Pacific, and one endemic *Cereus* species occurs on the black lava cliffs of Fernando de Noronha in the Atlantic east of the coast of northeastern Brazil (Barthlott, 1979). Cacti are often conspicuous elements of dry landscapes in the Americas and may be the dominant species in some communities. Of the three centers of diversity and endemism for the family the most outstanding is *Megamexico*, one of the global 'megadiverse' centers of biodiversity, an area that encompasses the nation of Mexico together with the southern portions of Texas, New Mexico, Arizona and California in the U.S. and the Guatemalan Highlands (Rzedowski, 1988; Myers et al., 2000). Here nearly half the species of the family are native, and more than ¼ of the genera and 1/3 of species are endemic (Taylor 1997). A second concentration of diversity of Cactaceae is centered in the Central Andes of South America, and a third Brazilian center represents the diversity of the epiphytic genus *Rhipsalis*.

The earliest literature of Cactaceae is attributed to the Caribbean historian Gonzalo Fernandez de Oviedo in 1526, and to descriptions of Mexican cacti in the catalogues of Francisco Hernández (Ximénez, 1615). Earlier illustrations survive in the archeological remains of pre-Columbian cultures: a painted mural at Teotihuacán, Mexico (*Opuntia*) dates from the 8th century A.D. and a petroglyph (*Trichocereus*) at the Chavin site in central Peru is dated to around 1300 A.D. (Rowley, 1997). In Mexico, cacti were depicted in Aztec glyphs and in the herbals destroyed by the Spanish conquerors (Sánchez-Mejorada, 1982). Naïve paintings illustrating the medicinal uses of two cacti are reproduced in the later Badianus Manuscript (de la Cruz, 1552). Following the Spanish conquest living specimens of globular and columnar forms of cacti were exported to Europe and thereafter began to appear in European literature by the late 16th Century (Anderson, 2000; Barthlott, 1979).

Towards a natural classification of Cactaceae

As species of cacti slowly came to be known in Europe during the 17th and 18th Centuries, a series of artificial classification schemes reflects gradual progress towards a modern phylogenetic classification. A summary of the history of Cactaceae classification was provided by Barthlott (1988, in German) nearly twenty years ago. At least four genera, *Cereus* Hermann (1698), *Opuntia* Tournefort (1700), *Pereskia* Plumier (1703), and *Melocactus* Tournefort (1719) were described shortly before Linnaeus (1737) organized the 16 known species of cacti into only two genera: *Pereskia* and *Cactus*[14]. Linnaeus (1753) grouped all 22 recognized species into a single genus *Cactus*, divided into four phenetic categories. More than 50 years later Haworth (1812) recognized 7

[14] According to Shaw (1976 cf. Anderson, 2001) *Cactus* may have originated from the shortening of *Melocactus*, an old name for a spiny plant. At the 1905 Botanical Congress in Vienna the name *Cactus* was rejected in favor of the later *Mammillaria*; the latter is now the conserved type of the family.

genera in Cactaceae[15]: *Cactus, Cereus, Opuntia, Pereskia, Rhipsalis,* and the newly described genera *Epiphyllum, and Mammillaria*. De Candolle (1828) divided 7 genera and 174 species of cacti into two artificial groups based on epiphytic vs. non-epiphytic habit. Famous for the living collection he amassed and concomitant catalogues resulting describing its species, the German Prince Salm-Reifferscheidt-Dyck (1850) divided the family into either rotate or tubulose flower groups. Within these 2 broad divisions however, Salm-Dyck recognized 20 genera that he arranged in 7 tribes based partially on natural characteristics but also on nomenclatural consistency.

The first monograph of Cactaceae was carefully crafted by Karl Schumann (1897). Schumann's division of the family into 3 natural subfamilies Pereskioideae Opuntioideae and Cereoideae (=Cactoideae) echoed that of Engelmann (1876). Division into these 3 natural groups at subfamily or tribal rank has been accepted by all workers since; only recently has DNA evidence (Crozier, 2004; this thesis) enabled slight refinement of these groupings. Schumann's Pereskioideae comprised only *Pereskia* and his Opuntioideae included *Opuntia, Nopalea* and *Pterocactus*. He divided Cereoideae into three tribes segregating the Rhipsalideae (monophyletic except for the inclusion of *Pfeiffera*), Mammillarieae (*Ariocarpus*, *Pelecyphora* and *Mammillaria*), and in Echinocacteae maintaining the polyphyletic collective genera *Cereus* and *Echinocactus* together with *Epiphyllum* and *Schlumbergera*. Though by this time more than 600 species of cacti had been described, Schumann recognized only the 20 genera of Salm-Dyck initially, later accepting *Wittia* also.

Berger's (1926; 1929) division of Cactaceae in 3 subfamilies transferred *Maihuenia* from Opuntioideae to Pereskioideae as Spegazzini (1926) had also done. Berger divided Cereoideae into only two tribes, Rhipsalidae and Cereeae (=Cacteae). He

[15] The name Cactaceae was first used to describe the family by Antoine Laurent Jussieu in 1789.

divided Cereeae into 4 tribes: Phyllocacteae (incl. *Epiphyllum* and *Disocactus*), Mammillarieae (=Schumann's), Cereinae (columnar *Cereus* and South American globular cacti *Echinopsis, Lobivia, Rebutia* and *Arequipa*), and Echinocactinae. However, Berger's generic concept was extremely conservative; he recognized only 41 genera, and he maintained the unnatural genera *Cereus* and *Echinocactus* despite his own view that *Echinocactus* was probably polyphyletic. Instead, he divided the very large *Cereus* into 18 subgenera. His attempts to reconstruct the phylogenetic relationships of genera by identifying fundamental shared characters are clear in the dendrograms and descriptions laid out in his 1926 book. His contribution undoubtedly influenced later efforts toward natural classification.

The four-volume encyclopedic work of Britton and Rose (1923-1929) resulted from a coordinated effort to explore cactus regions, visit herbaria, and document the diversity of Cactaceae. They recognized more than 1200 species in 124 genera, raising many of the subgenera of Berger to generic rank. Subfamilies Pereskioideae, Opuntioideae, and Cereoideae (=Cactoideae) were relegated to tribal rank. Seven genera were recognized in Opuntieae: *Pereskiopsis, Pterocactus, Nopalea, Tacinga, Opuntia, Grusonia* as well as *Maihuenia*. Cereeae genera were grouped into 8 subtribes: 1. Cereanae, a polyphyletic[16] assemblage encompassing all the columnar cacti (37 genera); 2. Hylocereanae a paraphyletic group comprising 9 genera; 3. Echinocereeanae, a polyphyletic group of species with low-growing usually single-jointed ribbed stems; 4. Echinocactanae a polyphyletic assemblage of 28 genera mostly with seeds dehiscing from a basal pore; 5. Cactanae, containing only the sister genera *Discocactus* and *Cactus* (*Melocactus*) that bear terminal cephalia; 6. Coryphanthanae, a paraphyletic

[16] Polyphyletic, paraphyletic, and monophyletic are terms coined in the 1960s by Willig Hennig; they are used here in retrospect.

group of 14 globose to short cylindrical tuberculate genera; 7. Epiphyllanae, a group of 9 night-blooming epiphytic genera; and 8. Rhipsalidanae, diphyletic group of 8 small rotate-flowered epiphytic genera. Britton and Rose are credited with laying a foundation for modern taxonomic treatments, though little of their classification or generic taxonomy was adopted by subsequent workers. A number of small so-called micro-genera accepted and/or erected by Britton and Rose remain controversial among plant taxonomists (e.g., *Mammillopsis, Cochemiea, Neobesseya, Bartschella, Phellosperma, Dolichothele, Ancistrocactus, Toumeya, Homalocephala, Echinomastus, Hamatocactus, Solisia*).

Curt Backeberg's (1958-1962) six-volume encyclopedia of cacti resulted from observations made in the field beginning in 1929, and from the large living collection he amassed. He never preserved any herbarium specimen from the plants upon which his taxonomic decisions were based (Benson 1969a in Zimmerman, 1985). Backeberg accepted many of the small genera of Britton and Rose and split the family into more than 3,000 species in more than 220 genera. However, more than 90% of the approximately 80 genera he described are invalidly published. His taxonomic organization is clear, but artificial, and largely abandoned in systematic accounts of the family. Backeberg treated the family in three subfamilies recognizing in Peireskioideae (Pereskioideae) both *Peireskia* (*Pereskia*) and *Rhodocactus* and raising a new tribe equivalent to *Maihuenia*. In Opuntiodeae he recognized 16 genera in 3 tribes. Backeberg's subdivision of Cereoideae (=Cactoideae) followed the division by de Candolle (1828) and Berger (1926) into 2 tribes based on epiphytic (in Hylocereeae) vs. terrestrial (in Cereeae) habit. Emphasizing geography he further divided Cereeae: the northern genera circumscribed in 'semitribe' Boreocereinae and the southern genera circumscribed in 'semitribe' Austrocereinae. Each of these was divided by growth form into 'subtribes' Boreocereinae and Boreocactinae in the north and Austrocereinae and

Boreocereinae in the south. Below these ranks his system groups genera in 'kin' (Sippe) and 'subkin' (Untersippe) groups based on superficial characteristics resulted in a plethora of suprageneric names.

A contemporary of Backeberg, Franz Buxbaum advanced a more natural classification influenced by his own careful morphological studies (Buxbaum, 1950; Krainz 1956-1976), especially of reproductive structures, and by a theoretical framework emphasizing the importance of neoteny in the evolution of cacti. Buxbaum (1953; 1958; Endler & Buxbaum, 1974) again divided the family into 3 subfamilies placing *Maihuenia* in the Pereskioideae, and recognizing 8 genera in the Opuntioideae (*Quiabentia, Pereskiopsis, Tacinga, Pterocactus, Marenopuntia, Grusonia,* and *Brasiliopuntia,* and *Opuntia (*synonymizing *Cylindropuntia, Tephrocactus, Corynopuntia, Micropuntia, Consolea, and Nopalea*). He divided the Cactoideae into 9 tribes. He considered Berger's Trichocerei to be polyphyletic and correctly segregated a North American columnar cactus clade Pachycereae, though his segregation of Echinocereeae makes both tribes polyphyletic, and his further subdivision of Pachycereae into 5 subtribes is unnatural. He also distinguished a clade of South American columnar genera, Trichocereae, from predominantly globular genera in his Notocacteae (unfortunately including *Astrophytum* in the latter). The Brownigieae, erected to accommodate *Browningia* (including *Gymnanthocereus* and *Rauhocereus*), and Cereeae circumscribing a cereoid core group (without *Melocactus, Discocactus,* or *Gymnocalycium*) are advancements over previous systems. Buxbaum's Leptocereeae however, can now be identified as a polyphyletic collection of several basal genera of South American Cactoideae (*Calymanthium, Neoabbottia, Neoraimondia, Armatocereus, Leptocereus, Eulychnia, Eriosyce, Samaipaticereus,* and *Rodentiophila*). He placed 32 epiphytic genera in Hylocereeae divided into 5 subtribes: Nyctocereinae, Hylocereinae,

Epiphyllinae, Disocactinae, and Rhipsalinae (incl. *Pfeiffera*), rejecting Berger's segregation of more primitive-flowered epiphytes. The North American globular cacti were placed in the Cacteae, much as Britton and Rose had already done.

Several adjustments to Buxbaum's classification were made by Gibson and Nobel (1986) as new genera came to light. They correctly moved *Astrophytum* to Cacteae and, following Barthlott (1979), moved *Melocactus* to Cereeae, and reduced Echinocereeae to comprise only *Echinocereus*. Again the epiphytic genera in Rhipsalidae were separated from the Hylocereeae, but placed within the Notocacteae.

David Hunt (1967) provided a pragmatic, unapologetically artificial classification that recognized 84 genera and nearly 2,000 species. The three major groups of Cactaceae were recognized at tribal rank with the Cacteae split into two subtribes Cereinae and Cactinae. Within Cereinae, Hunt circumscribed three groups: 1. most columnar cacti (27 genera), 2. epiphytes including Hylocereae and Rhipsalideae, and 3. South American trichocereoid[17] columnar and globular cacti. The Cactinae included all the remaining globular cacti, these distributed among three groups: 1. South American trichocereoid globular cacti (7 genera), 2. *Melocactus* and *Discocactus* (=Cactanae sensu Britton & Rose); and 3. North American globular cacti together with South American *Copiapoa* and *Gymnocalycium*.

Barthlott (1988) revised the classification of Buxbaum (1974) using new evidence from electron-microscope studies of seeds, and with emphasis upon the 90 accepted genera of Hunt and Taylor (1986). Three subfamilies were recognized with *Maihuenia* included in Pereskioideae. Opuntioideae was treated as containing only four genera: *Opuntia, Tacinga, Pterocactus,* and *Perskiopsis*. Cactoideae was divided into eight tribes. As with Berger (1929), the epiphytes were treated as diphyletic, and *Astrophytum*

[17] bearing narrow acute scales with axillary hairs of the receptacular tube

was placed with the Cacteae as Backeberg had suggested. (*Melocactus* is included in the Cereeae.) In Barthlott's system: 1. Hylocereeae (treated as subtribe by Buxbaum) is recognized as containing 6 genera; 2. Echinocereae (recognized instead of Buxbaum's Leptocereeae) is circumsciribed *Leptocereus, Echinocereus, Harrisia, Peniocereus* and *Acanthocereus*; 3. Pachycereeae (six genera) is paraphyletic; 4. Browningieae is a polyphyletic assemblage of 5 genera; 5. Cereeae (seven genera) is also paraphyletic; 6. Notocacteae is a polyphyletic assemblage of 22 genera; 7. Rhipsalideae is monophyletic recognizing 4 genera; and 8. Cacteae is monophyletic recognizing 23 genera.

For *The Families and Genera of Flowering Plants* Barthlott and Hunt (1993) significantly revised Barthlott's scheme for the Cactaceae only five years earlier. Several additional genera were recognized and one additional tribe added to Cactoideae. *Quiabentia* was recognized in Opuntioideae. Within Cactoideae the formerly large tribe Notocacteae was split. Fourteen genera previously included in Notocacteae were placed in a resurrected Trichocereeae, along with five additional genera. To the eight genera remaining in Notocacteae, *Neowerdermannia, Eriosyce,* and *Blossfeldia* were added. *Cipocereus* and *Stephanocereus* were recognized in Cereeae; *Stetsonia* included in Browningiae; and *Neobuxbaumia, Rathbunia, Polaskia,* and *Escontria* were recognized in Pachycereae. Cacteae was no longer divided into two subtribes, and *Aztekium* and *Ortegocactus* were additionally recognized. Only Echinocereeae and Rhipsalideae were unchanged.

Revision incorporating molecular evidence

The molecular foundation for the present classification comes from two chloroplast DNA sequence studies discussed in detail earlier: a three-gene family-wide study (Chapter 2) and a larger study that included sequences from three Group II introns (*trn*K, *rpl*16 and *rpo*C1) and four intergenic spacer regions (*trn*K-*psb*A, *rpo*20-*rps*12,

*trn*L-*trn*F, and *trn*T-*trn*L) (Chapter 3). Briefly, DNA sequences were prepared by standard methods and data were analyzed using multiple methods based on very different optimality criteria. Conclusions of relationship drawn here reflect the broad agreement of results between analyses. Relationships were inferred from strongly supported clades with one exception. The monophyly of *Pereskia* is strongly challenged by these molecular results. However, statistical support for relationships among Pereskia clades is lacking and therefore relationships remain equivocal. Significant nucleotide substitution rate heterogenity exists between pereskias, while some *Pereskia* rates are like those of Opuntioideae. Site-rate heterogeneity across the phylogenetic tree is a violation of statistical model assumptions made in the Bayesian analyses thus far conducted. Therefore, the traditional view of a monophyletic Pereskioideae is maintained in this classification until further analyses can be conducted. However, it must be said that these results, indeed, need not conflict with a view that resurrects the genus *Rhodocactus* whose type species is *Pereskia grandiflora*. As in many families, difficulty in circumscribing monophyletic groups arises from the awkward appearance of grades at the base of clades. Until far more is known about the molecular evolution of genetic systems, our understanding of the early divergence of the major clades of cacti will not be unequivocal.

RESULTS FROM PREVIOUS MOLECULAR STUDIES

Results from molecular studies (Chapters 2 and 3) are summarized in phylogenetic trees presented in Figures 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9. These analyses of chloroplast DNA sequence data provide strong evidence of major clades in Cactaceae and their relationships, altering the earlier view of Cactaceae as evolving along only three morphologically distinct lineages. These results precipitated a revision of Cactaceae classification (Chapter 5) that recognizes six subfamilies (Crozier,

2004): Pereskioideae, Maihuenioideae, Opuntioideae, Blossfeldioideae, Cactoideae and Rhipsalidoideae.

Phylogenetic relationships identified

Surprisingly, the enigmatic monotype *Blossfeldia* was found to be a link between the basal lineages Opuntioideae-*Pereskia-Maihuenia* and Cactoideae-Rhipsalidoideae, sharing many molecular synapomorphies with the former (see Chapters 2 and 5)[18]. Previously, morphological studies have consistently placed this taxon squarely within the Cactoideae (including Rhipsalidoideae), never before suggesting it could provide a link between the that group and the opuntioids and pereskioids. The taxonomic implications resulting from the phylogenetic position and distinctiveness of this lineage are discussed in detail in Chapter 5 and recognized in this classification.

A strongly supported dichotomy places the North American dwarf cactus clade Cactoideae as sister to a large clade including tribes Pachycereeae, Hylocereeae, Notocacteae, Rhipsalideeae, Trichocereeae, Browningieae, Leptocereeae, Cereeae and Calymantheeae along with *Copiapoa*. This large clade, containing at least 9 tribes, had not been recognized previously in morphological analyses. Based on analysis of *trn*K/*mat*K and the *trn*L-*trn*F intron Nyffeler (2002) also identified the large clade with 90% bootstrap support, but misidentified the group *as* the "core Cactoideae" when in fact the clade *is sister to* the core of Cactoideae containing the type species (see Chapter 5). Instead, Rhipsalidoideae is nomenclaturally appropriate and used here. Nyfeller did not note the evolutionary significance on this sister relationship; the Cactoideae is actually a much older lineage than previously thought. Prior to molecular analyses the Cactoideae

[18] This result was found concurrently in a blind study (Nyffeler, 2002) using the two of the same markers used in this dissertation study. This result was so unexpected that Dr. Nyffeler requested, and was sent, the *Blossfeldia* trnK/matK sequence from this study for comparison with his results prior to his publication.

(=Cacteae) had been thought to have been derived from somewhere within the Rhipsalidoideae, with most cases speculating the Notocacteae or Pachycereae (Zimmermann, 1985). Cactoideae is restricted to North America with the exception of one or two species along the northern coast of South America belonging to the derived genus *Mammillaria*. Without any other geographical connection to the basal taxa in the sister clade Rhipsalidoideae (e.g., *Copiapoa, Calymanthium*) in South America, and with an origin predating the Isthmus of Panama[19], the Cactoideae therefore is likely the result of long distance dispersal as Simpson and Neff (1985) have described for other plant disjunctions. Further, Cactoideae are one of the few examples of a successful radiation into the deserts of Mexico that is of South American origin.

Within Rhipsalidoideae two distinctive genera, *Copiapoa* and the monotypic *Calymanthium*, are found to be sister to a large unnamed clade comprising Rhipsalideae, Notocacteae, Leptocereeae, Hylocereeae, Pachycereeae, Cereeae, Browningieae and Trichocereeae. Two sister clades comprise these eight tribes; their strongly supported sister relationship has no precedent in either morphological or molecular studies. Distinct epiphytic clades Rhipsalideae and Hylocereeae have arisen in parallel in each of these clades. The nominal clade containing Rhipsalideae includes also Notocacteae, Browningieae, Cereeae, and Trichocereae along with *Frailea*, *Uebelmannia* and *Rebutia* that are each identified here as distinct lineages. However, the placement here of Browningieae is only marginally supported by low posterior probability in the Bayesian

[19] Evolutionary divergence times in Cactaceae were calculated using the penalized likelihood method implemented in the program r8s (v 1.50; Sanderson, 2002) for the most parsimonious tree resulting from a MP analysis with reduced taxon sampling (results not shown). In the absence of Cactaceae macrofossils calibration was accomplished by first estimating the divergence of Cactaceae on a tree inferred from combined analysis of atpB, rbcL and 18S sequence data downloaded from Genbank for 22 Caryophylales, (incl. *Pereskia aculeata*) and 8 outgroup families, fixing the divergence dates of Caryophyllalles, Dilleniaceae, and Polygonalles (Magallón & Sanderson, 2001) and Aizoaceae (Klak, Reeves & Hedderson, 2003). Standard errors for divergence times were calculated via simulation using Seq-Gen (v1.2.5, Rambaut & Grassly, 1997).

combined analysis and this signal was contributed primarily by the *rpo*B data. The sister clade comprises a grade from the independent lineages *Corryocactus* and *Neoraimondia* to Leptocereeae, Hylocereeae, Pachycereeae, sister to the clade comprising *Pfeiffera*, *Eulychnia,* and *Austrocactus* (unnamed). The derived tribe Pachycereeae consists of the North American columnar cacti comprised in sister subtribes Pachycereinae and Stenocereinae. The placement of *Carnegiea, Pachycereus, Lophocereus, Cephalocereus*, and *Neobuxbaumia* in Pachycereinae, and *Stenocereus, Myrtillocactus* and *Escontria* in Stenocereinae by Gibson and Horak (1978) and Gibson (1982) is confirmed. However, those authors excluded *Echinocereus* and *Peniocereus* from the tribe. We are in agreement with the conclusions of Anderson (2001) and Nyffeler (2002) to include *Echinocereus* in the Pachycereeae and we do not recognize Echinocereeae. In addition, our studies show *Echinocereus* belongs to the Stenocereinae.

Relationships among basal lineages

Basal relationships in Cactaceae between Opuntioideae (300+ species), *Maihuenia* (2 species) and *Pereskia* (17 species) remain uncertain. Opuntioideae is clearly monophyletic defined by numerous molecular synapomorphies. However, the relationships of Opuntioideae, *Pereskia,* and *Maihuenia* differ when different loci are analyzed and when different methods are used. Bayesian analysis of *rbc*L strongly favors Opuntioideae sister to Blossfelioideae-Cactoideae-Rhipsalidoideae and *Pereskia* united with *Maihuenia*. In contrast, analysis of *rpo*B strongly favors Opuntioideae as derived within a clade of some pereskias, while analysis of *mat*K cannot resolve with confidence the relationships between Opuntioideae, *Maihuenia* and several *Pereskia* branches.

Different gene trees may appear incongruent due to a variety of causes other than differing underlying organismal histories (Bull et al., 1993; Huelsenbeck et al., 1996). In Cactaceae basal relationships differ (e.g., Figures 2.4, 2.5, 2.8, 2.9) when methods based

on different optimality criteria were used. When different methods yield congruent tree topologies the results are generally accepted as robust. However, contrasting results can be compared to look for clues to the data characteristics (Sanderson, 2002a). In the present studies neither maximum parsimony nor Bayesian analyses resolved the relationship of all *Pereskia* species with confidence. The Bayesian analysis joined the *P. lychnidiflora*, *P. aculeata*, and three Andean pereskias, *P. horrida, P. diaz-romeriana, and P. weberiana* with the Opuntioideae having 100% posterior probability, but there was no bootstrap support for this relationship using maximum parsimony. The minimum evolution distance tree placed two clades of pereskias in a grade, with *Maihuenia* sister at the base of the family. *Pereskia* species appear to have diverged very rapidly early in the history of the family accumulating few synapomorphies for the genus. A parametric bootstrap hypothesis test of these data confidently rejected the null hypothesis of *Pereskia* monophyly when simulated data sets were modeled using the using the same model of evolution used in phylogenetic analysis (see Chapters 2 and 3). However, branch lengths differ significantly between the Andean *Pereskia* clade and other pereskias, yet are not significantly different between the Andean *Pereskia* clade and the Opuntioideae clade (Figure 2.5). Branch-length heterogeneity can increase the risk that homoplastic changes on long branches are erroneously interpreted as synapomorphies and outnumber changes on the short branches separating them, the infamous long-branch attraction problem (Felsenstein, 1978; Hendy & Penny,1989; Huelsenbeck & Hillis, 1993; Hillis, 1998; Phillipe, 2000) that is also difficult to detect (Huelsenbeck, 1997; Sanderson, 2002). In the absence of unequivocal molecular evidence, the traditional subfamilies Opuntioideae and Pereskioideae are maintained.

The classification of Barthlott & Hunt (1993) is revised here with new evidence from chloroplast DNA studies (this thesis) and published molecular studies (Nyffeler,

2001; Wallace & Dickie, 2002) to reflect a phylogenetic framework for Cactaceae. Several good genera are accepted that were not previously recognized by Barthlott and Hunt (1993) or Hunt (1999). *Blossfeldia* is a distinctly isolated lineage that does not share the loss of the *rpo*C1 intron with the Cactoideae, a molecular character widely considered to be a synapomorphy for the subfamily (Stevens, 2001 onwards [2005]) nor does it have the *trn*T-*trn*L intergenic spacer deletions described by Applequist and Wallace (2002) as important taxonomic characters in Cactoideae and Rhipsalidoideae. For nomenclatural reasons the name Cactoideae follows the genus *Mammillaria* so the name Rhipsalidoideae is resurrected to describe the sister clade containing *Rhipsalis* (Crozier, 2004). The three major clades comprising Rhipsalidoideae have no historical precedent and might be named at supertribe rank.

A REVISED CLASSIFICATION OF CACTACEAE

Cactaceae A. L. de Jussieu

Subfamily Pereskioideae Engelm. (1876)

Trees, shrubs or lianas sometimes with tuberous roots; stems not markedly succulent, terete; leaves broad, semisucculent, persistent, deciduous during dry season, areoles producing spines in each growing season. Flowers terminal, pericarpel areoles with trichomes or spines, sometimes with leaves persistent and soon deciduous after fruit maturation, tube absent, perianth parts red, yellow, orange, pink, purple, or white. Fruits indehiscent, fleshy. Seeds black or brown, shiny. Contains the *rpo*C1 intron. Tropical America from Mexico to northern Argentina absent from Ecuador, Chile and the Amazonian region.

Pereskia, 17 species

Subfamily Maihenioideae Fearn (1996)

Caespitose shrubs; stems succulent, globose or cylindric, leaves linear or terete, semisucculent, persistent, leaves produced below and within the areole, areoles with 3 spines. Flowers terminal, solitary, pericarpel areoles without spines, tube absent, perianth parts white or yellow, sometimes drying pink or brown. Fruits indehiscent, oblong, pericarpel walls fleshy, otherwise hollow. Seeds oblong to circular, black, shiny. Contains the *rpo*C1 intron. Southern Argentina and Chile.

Maihuenia, 2 species

Subfamily Opuntioideae Burnett (1835)

Erect trees or shubs, caespitose or scandent, stems segmented, succulent, subglobose, cylindric, or broadly flattened; leaves persistent or soon deciduous, linear or terete, semisucculent, areoles with multiple spines and glochids rarely absent, spines acicular or flattened and papery. Flowers lateral (rarely terminal), sessile, solitary, pericarpel areoles with sometimes with leaves, glochids, and spines, tube short or absent, rotate or sometimes tubular, perianth parts white, pink, orange, red, yellow, or purple, rarely brown. Fruits indehiscent, rarely dehiscent, juicy or dry accessory berries, round to oblong. Seeds oblong to circular, surrounded by a bony aril derived from the funiculus, the aril brown to creamy-white, sometimes pink or purple, sometimes

verrucose or winged. Chloroplast genome contains the *rpo*C1 intron. America, highest generic diversity in southern and western South America. 4 tribes:

Tribe Austrocylindropuntieae Wallace & Dickie (2002)

Shrubs, few-branched or forming dense clumps or dense mats, stems shallowly segmented with basitonic or mesotonic growth, areoles with spines, sometimes with pectinate spines, glochids and trichomes, rarely glochids absent; leaves succulent, persistent or deciduous, primary spines lacking leaf-sheath. Seeds with hook-shaped or ring embryos, perisperm reduced or prominent. Andean South America, from Ecuador to Argentina.

Austrocylindropuntia Backeb. 9-10 species

Cumulopuntia F. Ritter 18-20 species

Maihueniopsis Speg. 13 species

Tribe Cylindropuntieae Doweld (1999)

Trees or shrubs with cylindric stems, stems segmented with acrotonic growth, primary stems monopodial, caespitose or solitary; leaves persistent or deciduous, sometimes flattened lamina, areoles with acicular or flattened spines, primary spines with sheaths. Seeds with circular, rarely spirally arranged, embryos. USA and Mexico, naturalized in Argentina, Chile and Bolivia.

Cylindropuntia (Engelm.) F. Knuth 35 species

Grusonia F. Rchb. ex Britton & Rose 17 species

Pereskiopsis Britton & Rose 7-8 species

Quiabentia Britton & Rose 2 species

Tribe Opuntieae

Trees, scandent or erect shrubs sometimes with dimorphic growth, sometimes forming extensive patches, stems acrotonic, stems flattened or rarely cylindric, areoles with spines, trichomes and glochids, rarely without spines; leaves terete or ovoid, soon deciduous. Flowers sometimes nearly completely closed with only stamens and style protruding from perianth parts. Fruits sometimes dehiscing by a lateral split Seeds with circular to hook-shaped, or sometimes with spirally arranged embryos, perisperm reduced. America.

Brasiliopuntia (K. Schum.) A. Berger 1 species

Consolea Lem. 7 species

Miqueliopuntia Fric ex F. Ritter 1 species

Opuntia Miller 150 species

Tacinga Britton & Rose 6 species

Tunilla D. Hunt & Iliff 12 species

Nopalea Salm-Dyck 10 species

Tribe Pterocacteae Doweld (1999)

Articulated, geophytic plants, stem segments globular or cylindric, roots tuberous, areoles with spines and glochids, sometimes glochids absent; leaves minute,

deciduous. Flowers yellow or red. Fruits dry, capsular, dehiscing laterally. Seeds brown, embryo ring-shaped, perisperm prominent. Argentina.

Pterocactus K. Schum. 9 species

Tribe Tephrocacteae Doweld (1999)

Small shrubs, pseudocaespitose, with multiple cylindric, or round segments, branching terminal or subterminal, areoles with trichomes, glochids, and spines, spines sometimes absent; leaves minute, caducous. Flowers white or pink, yellow, red. Fruits dry, dehiscent. Seeds cream to brown, embryos hook-shaped, perisperm prominent. Argentina

Tephrocactus Lem. 6-7 species

Subfamily Blossfeldioideae Crozier (2004)

Perennial herbs from a fleshy taproot, succulent, poikylohydric, body swelling immediately after rainfall. Stem solitary or caespitose, individual stems obpyriform when hydrated or flattened disc-shaped with conspicuous central cup-like depression when dessicated, lacking ribs or pronounced tubercles, 1.0-1.5 (2.5) cm in diameter. Stomata restricted to areolar pits, overall density much less than 1 per mm^2. Pericarpel sculptured with podaria tipped by small lanceolate to triangular scales, or with only a few scales and essentially glabrous on the lower part, bearing whitish to gray wooly hairs in the axils. Pollen subspherical, tricolpate, with smooth exine. Fruit a juicy accessory

berry, spherical to ovoid or pyriform, about 0.5 cm. across with podaria bearing large scales, and axillary hair in small bundles, without bristles, side splitting when ripe then disintegrating over time to release the seed. Seeds globose, small, ca. 0.5 mm in diameter, testa minutely papillose, shiny red-brown, with large ivory hilum. Chloroplast genome contains the *rpo*C1 intron. Northern Argentina, southern Bolivia,

Blossfeldia Werderm. 1 species

Subfamily Rhipsalidoideae Burnett (1835)

Trees or shrubs with erect or decumbent stems or sometimes reduced to small, globular, caespitose or solitary stems, sometimes scandent and epiphytic or supported by neighboring vegetation, roots fibrous or tuberous, stems usually unsegmented, ribbed, sometimes tuberculate, fertile undifferentiated or differentiated, cephalia apical or lateral; leaves vestigial, areoles with spines, bristles, trichomes, without glochids. Flowers diurnal or nocturnal, nocturnal flowers sometimes very large, invariably white or creamy white, diurnal flowers of various colors, mostly white, pink, yellow, or red, rarely purple-blue, pericarpel usually areolate or with scales, sometimes naked, sometimes areoles producing dense tufts of wooly trichomes and covering pericarpel, ovary inferior. Fruit a juicy or dry accessory berry, globose, ovoid, pericarp fleshy or dry, indehiscent or dehiscent. Seeds various, normally oval to circular, black or brown, aril absent. Loss the *rpo*C1 intron. America

Tribe Calymmanthieae Wallace nom. nud.?

Shrubs or trees, stems segmented with 3-4 ribs. Flowers with tube partially covering the perianth in bud, pericarpel areoles with trichomes and soft spines, pericarpel red distally. Fruit large, fleshy, indehiscent. Seeds oval, covered with mucilage sheath. Northern Peru

Calymmanthium Ritter 1 species

Tribe Copiapoeae (Doweld) Doweld (2002)

Solitary or caespitose, mound-forming shrubs, stems globose to cylindric, ribbed and tuberculate, apices covered with dense wool, stems green or sometimes bluish white, spines few to many, either white or dull black. Flowers short-campanulate, yellow or golden-yellow sometimes suffused with pink, rarely reddish orange, pericarpel essentially glabrous. Fruits globose to cylidric, dehiscent at apex. Chile, Atacama desert and adjacent matorral area to the south, in proximity to the ocean.

Copiapoa Britton & Rose 20 species

Austrocactus clade (unnamed)

Shrubs, sometimes tree-like or variously small low growing solitary, globose, simple or branched stems or epiphytic with cylindric stems, stems ribbed rarely pseudotuberculate, areoles with minute to very long spines, sometimes hooked. Flowers subapical or lateral, pericarpel segments soft pink or white, rarely yellow or orange, stigmas white or sometimes deep purple, pericarpel sometimes with multiple, minute bracts, obconic, areoles with blackish, woolly trichomes or sometimes spiniferous. Fruits dry or fleshy and very juicy, splitting irregularly or at base. Seeds oval. Argentina, Bolivia, and Chile.

Austrocactus Britton & Rose 5 species

Eulychnia Philippi 6 species

Pfeiffera Salm-Dyck 3-5 species

Corryocactus clade (unnamed)

Large shrubs or sometimes arborescent, stems erect or procumbent, ribs 4-10. Flowers campanulate, diurnal, areoles of preicarpel felted, spiny, pericarpel segments yellow or orange, sometimes red. Fruits globose, a large berry with numerous seeds. Seeds oval. Peru, Bolivia, and Chile.

Corryocactus Britton & Rose 20 species

Neoraimondia clade (unnamed)

Shrubs or trees, ribs 4-8, areoles large, knob-like, brown, felted, spinescent with one spine normally longer than others, those producing flowers essentially spineless, and longer than vegetative ones, flowering occurring on some areoles annually. Flowers deep pink or white, pericarpel areoles without spines, sometimes with bristles. Fruits round. Seeds oval, with mucilage. Peru, Bolivia, and Chile.

Neoraimondia Britton & Rose 2 species

Tribe Leptocereeae F. Buxb. (1958)

Trees, shrubs, scandent or semiprostrate, stems segmented, ribbed, ribs 3-8, spines acicular. Flowers white, yellow, or pink, pericarpel areoles with spines. Fruits globose to oblong, fleshy. Seeds black. Antilles.

Leptocereus (A. Berger) Britton & Rose 12 species

Tribe Hylocereeae (Britton & Rose) F. Buxb. (1958)

Scandent or epiphytic shrubs with aerial roots, stems with a few ribs or flattened. Flowers lateral, white, pink, or red, pericarpel areoles glabrous or with spines. Fruit a berry, indehiscent. Seeds with hilum and micropyle fused. Tropical America, mainly Central America

Disocactus Lindley 20 species

Epiphyllum Haw. 15 species

Hylocereus (A. Berger) B. & R.16 species

Pseudorhipsalis Britton & Rose 5 species

Selenicereus (A. Berger) Britton & Rose 20 species

Weberocereus Britton & Rose 9 species

Tribe Pachycereeae F. Buxb. (1958)

Tree-like, forming massive, candelabra-like structures or small cylindrical plants, either with erect or scandent stems, sometimes with massive storage roots, floral zone with or without cephalia, cephalia lateral or apical. Flowers lateral or restricted to the cephalium, diurnal or nocturnal, medium to large size, sometimes very showy, white, pink, yellow, green, or red, mostly actinomorphic, rarely zygomorphic, pericarpel with areoles or scales, pericarpel usually spiny or with soft bristles, rarely glabrous. Fruits globose, fleshy, spiny, scaly or sometimes glabrous, indehiscent or dehiscent. Seeds various. Most species in Mexico, a few in the USA, Caribbean and northern South America, a few species in eastern South America.

Acanthocereus (A. Berger) Britton & Rose 6 species

Carnegiea Britton & Rose 1 species

Cephalocereus Pfeiffer 3 species

Echinocereus Engelm. 50 species

Escontria Britton & Rose 1 species

Harrisia Britton 20 species

Pachycereus (A. Berger) Britton & Rose 13 species

Myrtillocactus Console 4 species

Neobuxbaumia Backeb. 7 species

Peniocereus (A. Berger) Britton & Rose 22 species

Stenocereus Riccobono 28 species

Frailea Clade (unnamed)

Low shrubs, caespitose or solitary, stems globose to cylindric, weakly ribbed or tuberculate. Flowers funnelform, yellow, diurnal, areoles of pericarpel densely pubescent and with bristles. Fruits dry, indehiscent or splitting irregularly. Seeds oval or with hilum depressed and C-shaped. Bolivia, Brazil, Paraguay, Uruguay, and Argentina.

Frailea Britton & Rose 15 species

Tribe Rhipsalideae DC. (1828)

Epiphytic or rupicolous, usually pendulous or sometimes tightly wrapping around stem branches, stems terete or angled, normally segmented. Flowers mostly lateral, rarely apical, pericarpel white, yellow or pink, pericarpel usually glabrous, rarely with areoles. Fruit a berry, indehiscent. Seeds with hilum and micropyle fused. America, most species diversity in Bolivia and Brazil, one species of *Rhipsalis* from eastern Africa to Sri Lanka.

Rhipsalis Gaert. 50 species

Hatiora Britton & Rose 3 species

Tribe Notocacteae F. Buxb. (1958)

Globular mostly small, rarely with very large globular to round-columnar stems, stems unsegmented, ribbed, ribbed-tuberculate or tuberculate, without cephalia but sometimes apical growth area covered with dense trichomes, rarely apical area depressed or sunken. Flowers apical, diurnal, yellow, red, orange or vivid shades of pink, actinomorphic, rarely zygomorphic, pericarpels covered with numerous scales, sometimes bristle-tipped and densely lanose. Fruits dry, dehiscing laterally or through a basal pore, rarely indehiscent, mostly dry and hollow, sometimes bright pink, balloon-like, indehiscent. Seeds broadly oval to circular, mostly ruminate. Pacific coast of Peru and Chile, Bolivia, Argentina, Paraguay, Uruguay, and Brazil.

Eriosyce Philippi 30 species

Parodia Speg. approximately 50 species

Neowerdermannia Backeb. 2 species

Yavia Kiesling & Piltz 1 species

Rebutia clade

Globular or cylindric, low stems, simple or caespitose, areoles circular or oval, sometimes nearly linear, spines soft. Flowers diurnal, funnelform, red, pink, yellow, orange and multiple hues in between, pericarpel with glabrous or pubescent scales. Fruits subglobose, dry. Seeds oval. Bolivia to Argentina.

Rebutia Schumann approximately 50 species

Uebelmannia clade

Globose, unbranched stems, ribbed, epidermis sometimes covered with wax areoles with soft or strong spines. Flowers subapical, funnelform, yellow, pericarpel areoles densely pubescent, woolly, sometimes with soft spines or bristles. Fruits dry, globose, with a rim of bristles at apices. Seeds oval, shallowly verrucose. Brazil

Uebelmannia Buining 5 species

Tribe Browningieae F. Buxb. (1966)

Trees or shrubs, or small globose stems, stems cylindric , ribbed, ribs 7-34, areoles with dense trichomes, brown, circular, with soft spines, flowering areoles with less spines, rarely tuberculate. Flowers tubular, nocturnal or diurnal, tube curved, perianth gray distalmost perianth segments white, red, or yellow (diurnal), pericarpel areolate, areoles glabrous, rarely naked. Fruits ovoid, fleshy, mucilaginous. Seeds black, smooth to ruminate. Peru, Bolivia, Chile.

Browningia Britton & Rose 7 species

Cintia Riha 3 species

Tribe Cereeae Salm-Dyck (1840)

Trees or shrubs, sometimes globular usually much-branched and forming large candelabra-like trees, rarely caespitose, stems unsegmented, sometimes segmented, ribbed, areoles spiniferous, rarely unarmed, fertile zone sometimes differentiated by a cephalium, the cephalium and fertile zone either lateral or apical. Flowers regular small to very large, fleshy, nocturnal white or diurnal, white, pink or cherry-red, pericarpel essentially naked, sometimes with thickened scales. Fruit and indehiscent berry, floral remnants usually persistent. Seeds oval, essentially smooth. Mostly eastern South America, extending to the southern Andes of Bolivia. *Melocactus* reaching Peru, the Caribbean, Mexico, and Central America.

Arrojadoa Britton & Rose 3 species

Brasilicereus Backeb. 2 species

Cereus Miller 35 species

Cipocereus Ritter 5 species

Coleocephalocereus Backeb. 6 species

Discocactus Pfeiffer 8 species

Gymnocalycium Pfeiffer approximately 50 species

Melocactus Link & Otto 32 species

Micranthocereus Backeb. 9 species

Pilosocereus Byles & Rowley 35 species

Stetsonia Britton & Rose 1 species

Stephanocereus A. Berger 2 species

Tribe Trichocereeae F. Buxb. (1958)

Tree-like or with a few massive stems several meters tall, stems columnar sometimes prostrate, solitary or caespitose, ribbed, sometimes tuberculate, fertile area lateral sometimes with cephalia. Flowers lateral, sometimes very large diurnal or nocturnal, white to soft pink, bright pink, rarely red, pericarpels with many scales and pubescent areoles sometimes with many black, woolly trichomes covering most of the base of the flower except for terminal pericarpel segments, actinomorphic, sometimes zygomorphic and closed with only the style protruding beyond the flower. Fruit a juicy or dry accessory berry, splitting irregularly. Seeds brown or black. South America

Arequipa B. & R. 2-3 species

Arthrocereus (A. Berger) A. Berger 5 species

Brachycereus Britton & Rose 1 species

Cleistocactus Lem. 40-5 species

Denmoza Britton & Rose 1 species

Echinopsis Zucc. 80-100 species

Espostoa Britton & Rose 10 species

Espostoopsis F. Buxb. 1 species

Facheiroa Britton & Rose 3 species

Haageocereus Backeb. 40 species

Lasiocereus Ritter 2 species

Leocereus Britton & Rose 1 species

Matucana Britton & Rose 20 species

Mila Britton & Rose 1 species

Oroya Britton & Rose 2 species

Oreocereus (A. Berger) Riccobono 3-4 species

Samaipaticereus Cardenas 1 species

Weberbauerocereus Backeb. 7 species

Subfamily Cactoideae Eaton (1836)

Globular solitary or caespitose stems, sometimes columnar, roots fibrous or napiform, stems unsegmented, ribbed, ribbed-tuberculate. or tuberculate, sometimes with compound ribs, areoles oval, sometimes grooved or divided with the spinescent areole on the distal part of the tubercle and the floriferous areole at the axis of the stem, fertile zone undifferentiated. Flowers subapical or apical, diurnal, actinomorphic, sometimes zygomorphic, white, pink, red, yellow, orange; pericarpel, scaly or naked, scales glabrous or densely pubescent, sometimes scales subulate, acicular. Fruit a berry, fleshy, rarely dry, indehiscent or dehiscent. Seeds oval to circular, rarely hat-shaped, testa smooth, verrucose or pitted, hilum and micropyle disjunct, rarely conjunct. The *rpo*C1 intron absent from chloroplast genome. North America a few species extending to Central America, the Caribbean, and northern South America.

Geohintonia clade

Plants solitary, sometimes clustering, globose to somewhat columnar, apices woolly, stems, ribbed, ribs sometimes with grooves, forming compound ribs, areoles woolly when young soon glabrous, spines 1-3, deciduous. Flowers borne at apex of stem, diurnal, actinomorphic, white, whitish-pink, deep pink. Fruit an accessory berry,

embedded in the apical tomentum, dehiscent, splitting irregularly. Seeds oval, tuberculate, shiny black. Northeastern Mexico.

Aztekium Boedeker 2 species

Geohintonia Glass & W. A. Fitz Maurice 1 species

Tribe Echinocacteae K. Schum. (1898)

Plants solitary globose to columnar, sometimes caespitose rarely tuberculate, stem apices with a large area of woolly trichomes from which flowers arise, glabrous or covered with tufts of hairs or scales, ribs 4-10, areoles large, distinct or confluent, spines present or absent. Flowers apical, diurnal, funnelform, yellow, sometimes with a red throat, pink, orange, pericarpel scales bristle-tipped, sometimes densely pubescent. Fruits globose, scaly, dehiscent, splitting irregularly. Seeds oval to circular, hat-shaped because of sunken hilum, smooth or tuberculate. Northeastern Mexico.

Astrophytum Lem. 5 species

Digitostigma Velazco & Nevárez 1 species

Echinocactus Link & Otto 6 species

Tribe Cacteae

Globular solitary or caespitose stems, sometimes columnar, roots fibrous or napiform, stems unsegmented, ribbed, ribbed-tuberculate or tuberculate, areoles oval,

sometimes grooved or divided with the spinescent areole on the distal part of the tubercle and the floriferous areole at the axis of the stem, fertile zone undifferentiated. Flowers subapical or apical, diurnal, actinomorphic, sometimes zygomorphic, white, pink, red, yellow, orange, pericarpel scaly or naked, scales glabrous or densely pubescent, sometimes scales subulate, acicular. Fruit an accessory berry, fleshy, rarely dry, indehiscent or dehiscent. Seeds oval to circular, testa smooth, verrucose or pitted. North America with a few species extending to Central America, the Caribbean, and northern South America.

Acharagma (N. P. Taylor) Glass 2 species

Ariocarpus Scheidweiler 5 species

Cochemiea (K. Brandegee) Walton 10 species

Coryphantha (Engelm.) Lem. 45 species

Cumarinia F. Buxb. 1 species

Epithelantha Britton & Rose 1 species

Escobaria Britton & Rose 13 species

Escobariopsis Doweld 1 species

Ferocactus Britton & Rose 25 species

Glandulicactus Backeb. 1 species

Lepidocoryphantha Backeb. 1 species

Leuchtenbergia Hooker 1 species

Lophophora J. Coulter 2 species

Mammillaria Haw. 150 species

Mammilloydia F. Buxb. 1 species

Neobesseya Britton & Rose 2 species

Neolloydia Britton & Rose 2 species

Obregonia Fric 1 species

Oehmea F. Buxb. 1 species

Ortegocactus Alexander 1 species

Pediocactus Britton & Rose 6 species

Pelecyphora Ehrenberg 2 species

Sclerocactus Britton & Rose 20 species

Strombocactus Britton & Rose 1 species

Thelocactus Britton & Rose 12 species

Turbinicarpus (Backeb.) F. Buxb. & Backeb. 10 species

Stenocactus (K. Schum.) A. W. Hill 10 species

Chapter 5: Subfamilies of the Cactaceae

INTRODUCTION

Taxonomic history

As in many other families of flowering plants there has been little unanimity in the suprageneric classification of the Cactaceae Juss., but slowly the discovery of new taxa, careful morphological observation, and other contributions to phylogenetic knowledge have led to refinements. The Cactaceae (Cacti) of Jussieu (1789) encompassed all the known cacti of the time under the single genus *Cactus* L., but also included *Ribes* L. (Grossulariaceae). Apparently aware of studies by de Candolle (1828) and Lindley (1830) that excluded Grossulariaceae from Cactaceae, Eaton (1836) nonetheless chose to divide Jusssieu's Cactaceae into two subfamilies, distinguishing Cactoideae (Cacteae) from Grossularieae. The Grossulariaceae were not included in Burnett's (1835) concept of Cactaceae (Nopalaceae) divided into subfamilies Rhipsalidoideae (Rhipsalidae) including only the genus *Rhipsalis* Gaertner, and Opuntioideae (Opuntidae) including the genera *Mammillaria* Haw., *Melocactus* Link & Otto, *Echinocactus* Link & Otto, *Cereus* Mill., *Opuntia* Mill., and *Pereskia* Mill. Engelmann's (1876) creation of subfamily Pereskioideae (Peireskieae) and division of the family into three subfamilies for the *Botany of California* begins the modern era in cactus classification. Treating only a few genera, Engelmann proclaimed the classification in three subfamilies years ahead of Schumann (1890,1898) whose Cactoideae (Cereoideae), Pereskioideae (Peireskioideae), and Opuntioideae have been included in most subsequent taxonomic studies (Berger 1926,1929; Backeberg 1958,1966; Buxbaum 1958, Barthlott & Hunt 1993; but see also Britton & Rose 1919-1923; Hunt 1967; Benson 1982 for

recognition of these same groups at tribal rank). Cactoideae Eaton, Opuntioideae Burnett, and Pereskioideae Engelm. appear to be validly published, and under the International Rules of Botanical Nomenclature (Greuter et al., 2000) these names take priority over the subfamilial names authored by Karl Schumann.

Molecular evidence for cladistic classification

The Opuntioideae and Pereskioideae have been clearly circumscribed and almost uniformly recognized in modern times, discounting the uncertain placement of *Maihuenia* Schum., a genus of only two species from the southern Andes and Patagonia. First associated with the caespitose opuntias, *Maihuenia* was soon reassigned to the Pereskioideae by Schumann (1898) based on spine, flower, and seed characters. Gibson's (1977) interpretation of stem tissues and pollen features supported this placement, however Bailey (1968) excluded *Maihuenia* from the Pereskioideae based on stem and vascular anatomy, and was also unwilling to place it with Opuntioideae based on similar terete leaves. Bailey noted similarities of pollen and highly modified wood that to him suggested a possible relationship with the Cactoideae (Cereoideae). Later, the genus was raised to subfamilial rank by Fearn (1996), who may have been spurred by provisional molecular evidence (see Leuenberger 1997 p. 58, and references within). With exclusion of *Maihuenia,* the monophyly of the Pereskioideae, including only *Pereskia* Mill., has never been questioned on morphological grounds. However, the molecular study of Nyfeller (2002) was unable to support the monophyly of *Pereskia*. A broader sample of six *Pereskia* species in the course of the present study did form a monophyletic clade with moderate bootstrap support in a maximum parsimony tree and significant probability in a Bayesian analysis. However, some partitions and methods used in the final study favor a polyphyletic *Pereskia* (see Chapter 2). The Pereskioideae is maintained here for the time being, until more complex evolutionary models for the

molecular data are explored, and basal relationships in the family can be reconstructed with more confidence. Together the Opuntioideae, Pereskioideae, and Maihuenioideae represent less than 15% of the species of the family. The rest of the family, a morphologically diverse group of more than 1100 species (Hunt 1999), has traditionally been lumped into the single subfamily Cactoideae based on the absence of synapomorphies defining the Pereskioideae and Opuntioideae. This diversity has usually been subdivided into 8 or 9 tribes (see Barthlott 1988 for a review; Barthlott & Hunt 1993). However, comparative analyses of chloroplast DNA sequence data now provide statistically well-supported evidence of two distinct major lineages. The name Rhipsalidoideae Burnett can be used to recognize the clade containing most columnar cacti, epiphytes, and globular cacti of South America.

Molecular studies are rapidly increasing our recognition of monophyletic lineages in the Cactaceae facilitating improved classification that reflects evolutionary relationships. Results of parsimony analysis of nearly 13,000 base pairs of chloroplast DNA sampled from representative taxa across the family first revealed *Blossfeldia* as a monophyletic lineage sister to the Cactoideae-Rhipsalidoideae clade with strong bootstrap support (Crozier & Jansen, 2001). Nyfeller (2002) independently inferred the same position of *Blossfeldia* rejecting the possibility that this might be a taxon-sampling artifact (long branch attraction) in parsimony and maximum likelihood combined analysis of *trn*K-*mat*K and *trn*L-*trn*F data. The present study compares 157 species of cacti, and outgroups from the Portulacaceae and Didieraceae for nearly 13,000 base pairs of chloroplast data using parsimony analysis that yielded strong bootstrap support for the *Blossfeldioideae* as well. Furthermore, statistical support for this relationship of *Blossfeldia* was 100% probable in a Bayesian analysis run for 4 million generations of that combined data set representing 10 functional regions of the chloroplast, including

genes, introns and intergenic spacer regions. In addition, *Blossfeldia* shares with the Pereskioideae, Opuntioideae and Maihuenioideae unique chloroplast DNA motifs in multiple markers. In particular, it does not share the *rpo*C1 intron deletion recognized as a synapomorphy for the Cactoideae (Wallace & Cota, 1985; Nyffeler, 2002; Stevens, 2001 onwards) nor the *trn*T-*trn*L intergenic spacer deletions described by Applequist & Wallace (2002) as important taxonomic characters in Cactoideae. For this reason and its distinctive morphology I placed *Blossfeldia* in its own subfamily (Crozier 2004).

Blossfeldioideae Crozier

Type: *Blossfeldia* Werd., Kakteenkunde 11:162 (1937).

Monotypic (1 species). Type species: *B. liliputana* Werd. Kakteenkunde 11:162 (1937).

Caudex crassus, caulis simplex dein proliferans, depresso-globosis vel disciformibus 2.5 cm diametro vel parvioribus, neque costatis neque tuberculatis vertice depressioribus lanoso, sine hypodermata epidermata una tabulato sine epicuticulo ceracea, parietibus cellularum epidermis externus vix incrassatis, stomata perpauci 1 per mm^2, stomata fovea areolari restrictis. Semina parva globosa strophiolo fere quam grandiore quam semina gerentibus.

Perennial herb from a fleshy taproot, succulent, poikylohydric, body swelling immediately after rainfall. Stem solitary or caespitose, individual stems obpyriform when hydrated or flattened disc shaped with conspicuous central cuplike depression when dessicated, lacking ribs or pronounced tubercles, 1-1.5 (2.5) cm in diameter. Stomata restricted to areolar pits, overall density much less than 1 per mm^2. Pericarpel sculptured with podaria tipped by small lanceolate to triangular scales, or with only a few scales and essentially glabrous on the lower part, bearing whitish to gray woolly hairs in the axils. Pollen subspherical, tricolpate, with smooth exine. Fruit a juicy berry, spherical to ovoid

or pyriform, ca. 0.5 cm. across with podaria bearing large scales, and axillary hair in small bundles, without bristles, side splitting when ripe then disintegrating over time to release the seed. Seeds globose, small, 0.5 mm in diameter, testa minutely papillose, shiny red-brown, with large ivory hilum.

At the present time the international rules of botanical nomenclature do not imply the nominal tribe, so a description is provided here.

Blossfeldia lacks xeromorphic stem features of other globular cacti (Barthlott & Porembski 1996) and its globose ornamented and arillate seed is distinctive in the family. Equally distinctive is the restriction of stomata to areolar crypts and extremely low density of stomata on the stem. Based on well-supported molecular analyses this smallest member of the Cactaceae represents an isolated lineage, and appears to be the only extant transitional form between the basal grade of subfamilies Pereskioideae-Opuntioideae-Maihuenioideae and a strongly supported clade of more derived cacti (see Chapter 2: Figure 2.6). At present no other member of the Blossfeldioideae has been identified.

Whereas distinct morphologies separate subfamilies Opuntioidae, Pereskioideae, Maihuenioideae and Blossfeldioideae, the remainder of species are so morphologically diverse that phyletic subdivision of the group has been difficult because of parallel and convergent evolution in vegetative and floral morphology (Buxbaum 1958; Barthlott & Hunt 1993). Classifications have been confusing and unstable, and circumscription of suprageneric taxa continues to be modified to try to meet modern expectations of monophyly (Buxbaum 1974, Gibson & Nobel 1986; I.O.S. 1986,1990; Barthlott 1988, Barthlott & Hunt 1993). In light of recent molecular studies a review of the entire suprageneric classification of the family, at least at the subfamilial level, seems in order.

The two lineages comprising the clade sister to Blossfeldioideae are quite distinct (see Chapter 2: Figure 2.6) and well supported by high bootstrap values and Bayesian

probabilities based on the chloroplast DNA studies in Chapters 2 and 3. These results show that the two groups are much more distantly related than are the groups of genera within each of them. It is appropriate to recognize these sister clades at equal rank. In so doing the information content of the classification is increased, and the adoption of six subfamilies is not so numerous as to negate its usefulness. Therefore, I am proposing that the proper application of the autonym Cactoideae belongs to the clade of North American globular cacti that includes *Mammillaria mammillaris* Haw., the conserved type species of the family. This clade corresponds to tribe Cacteae sensu Barthlott & Hunt (1993), though Backeberg (1966) may have been the first to recognize this monophyletic lineage with his subtribe Boreocactinae, a nomen nudum. The morphologically isolated position of this group from other tribes was pointed out specifically by Barthlott (1988) who noted "Zu allen übrigen Triben können keine Beziehungen erkannt werden." The earliest valid subfamilial name applicable to the sister clade of columnar, epiphytic and South American globular cacti appears to be Rhipsalidoideae Burnett. The Rhipsalidoideae as recognized here includes all members of tribes Rhipsalideae D.C., Echinocereae (Br. & Rose) Buxb., Hylocereae (Britton & Rose) Buxb., Cereae Salm-Dyck, Pachycereae Buxb., Trichocereae Buxb., Browningieae Buxb. and, with the exclusion of *Blossfeldia*, Notocacteae Buxb.

The Cactaceae has been notorious for parallel evolution in morphology that thwarts phylogenetic classification. Parallel reduction in shoot, leaf, flower and seed development in multiple phylogenetic lineages was described by Buxbaum (1951, 1956 and 1965) following phylogenetic ideas of Berger (1926). Although abundant molecular synapomorphies distinguish the Cactoideae and Rhipsalidoideae, unique morphological synapomorphies uniting each clade are difficult to identify. A key to the six subfamilies

recognized on the basis of morphological discontinuities and DNA evidence is provided below.

KEY TO THE SUBFAMILIES

1a. Areoles bearing glochids, seeds large, alveolate; bony aril covering the entire seed.……..……..**Opuntioideae.**

1b. Areoles without glochids, seeds small, usually exarillate or only the hilum covered by a strophiole or corky strophiolar pad.

2a. Plants with persistent photosynthetic leaves on stems.

3a. Plants tree-like or shrubs with laminar leaves ……..…….**Pereskioideae.**

3b. Plants low caespitose shrubs with terete leaves ..……...…….**Maihuenioideae.**

2b. Plants without persistent photosynthetic leaves on stems.

4a. Stems virtually lacking stomata except in sunken crypts; stems lacking thickened cuticle with epidermis lacking thickened outer cell walls, lacking thickened hypodermal layer, stem always flattened globular or disciform less than 25mm diameter; round seeds with strophiole nearly equal in size to the rest of the seed ....……..…….....……...**Blossfeldioideae.**

4b. Stems with stomata or if few then not restricted to cylindrical crypts, usually with thickened cuticle, an epidermal layer with outer cell walls thickened and a hypodermal layer; stems variously flat, triangular globular or columnar; seeds not round, seeds usually exarillate (except in *Parodia, Strombocactus,* and *Aztekium*).

5a. Flowers with naked pericarpels, bearing neither areoles nor scales.

6a. Plants globular or short cylindrical, never tall columnar, never with a cephalium, never epiphytic, restricted to North America or the Caribbean; seeds usually with disjunct hilum and micropyle ……....**Cactoideae.**

6b. Plants with flat (*Schlumbergera*), triangular, columnar (*Pachycereus, Pilosocereus, Espostoopsis*) stems or epiphytic (*Rhipsalis, Hatiora, Lepismium*), if globular then bearing a cephalium (*Melocactus*) or restricted to South America (*Uebelmannia*, some species of *Matucana*), seeds with conjunct hilum-micropylar region ……..…**Rhipsalidoideae.**

5b. Flowers with pericarpels bearing scales and/or areoles.

7a. Areoles on pericarpel naked.

8a. Stems globular or short cylindrical (*Astrophytum*, *Echinocactus*, *Sclerocactus papyracantha*) or barrel shaped (*Ferocactus, Echinocactus*), never epiphytic, seeds usually with disjunct hilum and micropyle, restricted to North America……..……..……...…….....…**Cactoideae.**

8b. Stems columnar or epiphytic, seeds always with conjunct hilum-micropylar region, American or Caribbean ……..……..……...……...**Rhipsalidoideae.**

7b. Areoles on pericarpel felted, or with hairs, bristles or spines……...…….....……...**Rhipsalidoideae.**

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Vita

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